

ADSA 2025 Annual Meeting

JUNE 22-25, 2025
LOUISVILLE, KENTUCKY

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Abstracts



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**Abstracts of the
2025 American Dairy Science Association®
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***Journal of Dairy Science*®
Volume 108, Supplement 1**





JOURNAL OF DAIRY SCIENCE[®] SINCE 1917

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American Dairy Science Association®
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Paul Kindstedt Recognition Symposium

1001 Cheese consistency and yield: From milk composition to cheese manufacture. J. J. Sheehan*, *Teagasc Food Research Centre Moorepark, Fermoy, Co. Cork, Ireland.*

Cheese ultimately is the product of the destabilization of casein micelle aggregates into chains and clusters, leading to formation of a 3-dimensional gel concentration of casein with subsequent dehydration and fermentation. Other macro- and micro-constituents (e.g., fat globules, water, minerals, bacteria, and dissolved solutes) are all interspersed within this casein matrix. There are many challenges to achieving optimum cheese yield and consistency during the manufacture process including that of the changing composition of milk. While traditionally changes to milk arose in certain regions due to seasonal production patterns, more recent dynamics include both the direct, and potentially indirect, changes to milk macro- and micro-constituents arising due to dairy breeding programs, as well as more atypical weather patterns with associated changes in herd diet. Similarly, recent changes to farming practices and initiatives driven by sustainability concerns may also affect milk composition and cheese manufacture. Research is currently focused on fingerprinting these changes in milk, in determining their impact on specific cheese manufacture process operations and ultimately on applying data analysis approaches and process control strategies to gain a deeper understanding of how commercial cheese manufacturers may achieve greater control in cheese moisture content and cheese yield management. The output of this research will be considered in the context of current knowledge.

Key Words: cheese yield, consistency

1002 Analysis of the physiochemical properties of mozzarella cheese: From melt characteristics to free oil and expressible serum. L. E. Metzger*, *Valley Queen, Milbank, SD.*

A cornerstone of Dr. Kindstedt's research program was the development and application of practical analysis methods for cheese and dairy products. The unique feature of Dr. Kindstedt's work was the clever utilization of simple, readily available laboratory equipment to evaluate and characterize critical physiochemical properties of cheese that provided insight into the impact of manufacturing parameters on cheese characteristics. Some examples of this work are (1) the application of helical viscometry for the analysis of mozzarella cheese stretch characteristics; (2) free oil measurement to predict the melt and browning characteristic of mozzarella cheese; and (3) expressible serum analysis to determine the ripening rate and optimal age for shredding and baking. These practical analysis methods played a central role in the understanding of the impact of manufacturing parameters on the functionality of mozzarella cheese and were critical for the rapid increase in production of mozzarella cheese between 1985 and 2005 when per capita consumption of mozzarella cheese doubled.

Key Words: Kindstedt, mozzarella

1003 The life of a pasta filata mozzarella scientist. S. Govindasamy-Lucey*, *Center for Dairy Research, University of Wisconsin–Madison, Madison, WI.*

Dr. Paul Kindstedt is one of the best-known scientists that have investigated the properties of mozzarella cheese. Mozzarella, especially the low-moisture, part-skim version, is the most popular cheese made in

the US and over the past 30 years has also become a globally important cheese variety. Its popularity is due to its widespread use in pizzas. Dr. Kindstedt undertook systematic studies on the impact of manufacturing parameters on the properties and performance of mozzarella. He worked closely with the cheesemakers to adjust their make recipes and with end users of the cheese in pizza restaurants to better understand their requirements. He developed various new methods to measure important parameters like the hot viscosity of melted cheese, free oil release on pizza, and state of protein during the baking process. Kindstedt and his collaborators at Cornell University did pioneering work in this area. They investigated manufacturing parameters such as culture types, rennet types, stretching temperatures, milling pH, low-fat versions, preacidification, late-lactation milks, fortification options, and many more. Dr. Kindstedt has spoken at many conferences and industry symposia over the years going as far afield as New Zealand. Dr. Kindstedt also did a sabbatical in University College Cork, Ireland, working with Dr. Fox on late-lactation milk on properties of mozzarella. He has published several highly cited reviews and book chapters on the properties of mozzarella cheese. The US industry has relied on this foundational work to greatly improve mozzarella functionality over the last years. There continues to be an increasing demand for cheese with very precise and targeted properties for stretch, melt, shredding, chewiness, and color. Dr. Kindstedt's foundational work has been critical in helping cheesemakers in tailoring cheese to meet these demanding performance requirements.

Key Words: pasta filata, mozzarella cheese, functionality

1004 Recognition of the contributions of Dr. Paul Kindstedt: Exploration of cheese crystals. G. F. Tansman*, *DSM-Firmenich, Princeton, NJ.*

Spanning over a decade of work, the Kindstedt laboratory at the University of Vermont produced innovative research into the formation of crystals in cheese during aging. The studies included elucidating mechanisms for crystal formation and the discovery of crystal phases not previously observed in cheese. Significant contributions included the development of image analysis techniques to characterize calcium lactate crystallization on Cheddar cheese, the characterization of 2 distinct chemical forms of calcium lactate crystallization in cheese, and the discovery of rare ikaite crystallization in washed rind cheese. The latter of these studies merited Kindstedt and his coauthors for the Hawley Medal by the Mineralogical Association of Canada for best publication of the year. This session highlights Dr. Paul Kindstedt's contribution to the field of dairy science through his studies of and expanded understanding of crystallization phenomena in cheese.

Key Words: Kindstedt, cheese, crystal

1005 Advances in the characterization of mozzarella cheese as influenced by Professor Paul Kindstedt's legacy. P. Sharma^{*1,2}, ¹*Nutrition, Dietetics and Food Science, Utah State University, Logan, UT,* ²*Western Dairy Center, Logan, UT.*

Mozzarella cheese is a pasta-filata style cheese widely consumed on pizza worldwide. The cheese has typical fibrous protein structures entrapping fat-serum channels forming a basis for pizza baking properties (i.e., meltability, oiling off, stretchability, and browning characteristics). To understand factors influencing these physicochemical characteristics of mozzarella cheese, it is important to have reliable,

consistent, and repeatable testing methods. Professor Paul Kindstedt took an early initiative in developing and fine tuning some of the critical testing protocols on mozzarella, which later became a stepping stone in developing advanced characterization tools using texture analyzers, rheometer, image processing, and microscopy. This presentation will highlight Professor Kindstedt's contributions to the cheese science that are especially important in the understanding and characterization of the physical and chemical properties of mozzarella cheese, as well as the processes that influence its texture, flavor, and quality. I will emphasize

particularly how his research on evaluating oiling off and melting functionality, understanding the role of calcium on cheese functionality, and modifying cheese-making technology revolutionized mozzarella cheese research and manufacturing practices worldwide. I will also describe how my research conducted at Massey University, New Zealand, on establishing useful links between rheology and functionality of mozzarella cheese was influenced by Professor Kindstedt's work.

Key Words: mozzarella cheese, functionality

Animal Health 1: Transition Cows

1100 Effect of *Solanum glaucophyllum*/Ca boluses administered shortly after calving on blood Ca concentrations in the first days of lactation. J. P. Goff*, *Accunutrition, Ames, IA.*

Solanum glaucophyllum produces a glycosylated form of bioactive 1,25-(OH)₂-vitamin D. *Solanum glaucophyllum* leaf plus Ca salt boluses (SG) were administered to Holstein cows within 6 h after calving and its effects on blood Ca concentration (BLCa) were compared with those of cows receiving no bolus or commercial Ca boluses in 2 studies. One study farm fed an anionic prepartum diet; one study farm did not. Cow treatments were no bolus (NoB), 2 SG boluses administered at calving only, or Ca bolus (43 g Ca) administered at calving and again 12–24 h later (CaBO). Blood was sampled before treatment and 3 (anion diet farm), 12, 24, 36, 48, 72, and 96 (No Anion diet farm) h later. Cows were assigned to treatments in each study based on parity and expected calving date. Effects of treatments on BLCa were analyzed using a repeated measures generalized linear mixed effects model. Cows were considered subclinically hypocalcemic at a time point if the average BLCa was below 2.00 mM. Results of anion diet herd: BLCa decreased from time 0 h concentrations at 3 h in the NoB cows ($n = 7$). In contrast, in cows receiving SG ($n = 10$) or CaBO ($n = 9$) BLCa had increased at 3 h. The BLCa continually increased in SG Bolus cows up to 48 h. The BLCa in CaBO cows peaked at 3 h. The BLCa of SG Bolus cows was significantly higher than in CaBO or NoB cows at 36 and 48 h after treatment ($P < 0.05$). From 24 to 72 h after treatment the SG Bolus group average BLCa was above 2.00 mM, whereas the average NoB and CaBO cow had BLCa < 2.00 mM from 24 to 72 h. Results of No Anion diet farm: BLCa of NoB ($n = 12$) and CaBO ($n = 12$) cows did not increase significantly from pretreatment concentrations until 72 h ($P > 0.05$). The SG cows ($n = 18$) had increased BLCa from pretreatment concentrations by 12 h ($P < 0.05$) and BLCa were increased above those of NoB and CaBO treated cows at 12, 24, 36, and 48 h ($P < 0.05$). The average BLCa of NoB and CaBO cows was < 2.00 mM until 72 h. SG Bolus cows had average BLCa > 2.00 mM from 36 h to 96 h. The SG boluses improved BLCa to a greater extent than commercial Ca boluses and reduced the length of time that blood Ca concentrations were below 2.00 mM in both studies.

Key Words: hypocalcemia, calcium bolus, *Solanum glaucophyllum*

1101 Effect of propylene glycol treatment on milk production in cows with hyperketonemia and varied blood parameters. M. R. Dorella*, E. Shepley, C. C. Fiorentino, M. Ruch, F. N. S. Pereira, J. N. C. Praxedes, E. M. Ribeiro Filho, A. G. M. Pereira, V. L. Junqueira, S. Giraldo, S. Davis, and L. S. Caixeta, *University of Minnesota, MN.*

This study evaluated whether nonesterified fatty acids (NEFA), glucose, or liver health index (LHI) values at the time of hyperketonemia (HYK; blood β -hydroxybutyrate [BHB] ≥ 1.2 mmol/L) diagnosis modified the effect of propylene glycol (PG) treatment on milk yield up to 120 DIM. Multiparous cows were screened for HYK twice between 2 to 8 DIM using a hand-held meter (Precision Xtra; Abbott Laboratories). The HYK-positive cows were randomly assigned to receive PG ($n = 229$) or no treatment (NoPG; $n = 217$). Plasma samples were analyzed for NEFA, glucose, bilirubin, albumin, and cholesterol using a small-scale chemistry analyzer (Chemwell-T, Catachem Inc.). The LHI was calculated from bilirubin, albumin, and cholesterol concentrations. Daily milk yield data were retrieved from farm records (Dairy Comp 305; Valley Agricultural Software). Effect modifiers were dichotomized as high

(NEFA > 0.72 mmol/L) or low NEFA, hypoglycemic (glucose < 40 mg/dL) or normoglycemic, and high (LHI > 0.13) or low LHI. Separate linear mixed models were used for each modifier and included treatment, modifier status at HYK diagnosis, relevant covariates, modifier-by-treatment interaction, and week of lactation as a repeated measure, with cow as a random effect. Regardless of treatment, milk yield did not differ by NEFA ($P = 0.14$) or glucose status ($P = 0.20$), but high LHI cows produced 3.2 ± 0.8 kg/d more than low LHI cows ($P < 0.01$). Among NoPG cows, milk yield was 44.7 ± 1.7 kg/d for low NEFA and 42.1 ± 1.7 kg/d for high NEFA cows ($P = 0.14$); in PG cows, yields were 42.4 ± 1.7 kg/d and 42.5 ± 1.6 kg/d, respectively ($P = 0.99$). Hypoglycemic NoPG cows produced 44.4 ± 1.8 kg/d and normoglycemic cows 43.1 ± 1.7 kg/d ($P = 0.73$); in PG cows, yields were 43.2 ± 1.8 kg/d and 42.3 ± 1.7 kg/d ($P = 0.87$). Low LHI cows produced less milk than high LHI cows in both NoPG (42.0 ± 1.6 vs. 44.6 ± 1.7 kg/d; $P = 0.08$) and PG groups (40.4 ± 1.7 vs. 44.2 ± 1.7 kg/d; $P < 0.01$). The treatment effect on milk yield up to 120 DIM was unaffected by NEFA, glucose, or LHI status at the time of HYK diagnosis; however, production varied with liver health.

Key Words: fresh cow management, ketosis, liver health index

1102 Metabolic and inflammatory responses to single and dual endotoxin challenges during negative energy balance in dairy cows. M. Chirivi*, U. Abou-Rjeileh, M. N. Myers, J. Parales-Girón, L. Worden, A. L. Lock, and G. A. Contreras, *Michigan State University, East Lansing, MI.*

Bovine periparturient diseases are often accompanied by endotoxemia leading to systemic inflammation and poor lactation performance. The effects of repeated inflammatory events on metabolism when cows are in negative energy balance (NEB) are still unknown. Our objective was to determine clinical and immune responses to endotoxemia induced by single or repeated intravenous (IV) lipopolysaccharide (LPS) challenges during NEB. Thirty-two multiparous Holstein cows (52.3 ± 5.5 kg/d of milk; 123 ± 42 DIM) were randomly assigned to treatment sequences. During period 1 (P1, d 1–7), baseline data were collected. In period 2 (P2, d 8–11), a feed restriction protocol was implemented to induce NEB (-14 Mcal/d). On d 10, cows were randomly assigned to receive either LPS ($1 \mu\text{g/kg BW}$, $n = 16$) or saline (SAL, $n = 16$). Period 3 (P3, d 12–18) served as a washout period. In period 4 (P4, d 19–22), a second feed restriction protocol was implemented, followed by a second IV infusion on d 21. Within each P2 treatment group (SAL or LPS), cows were re-randomized into one of 4 groups: (1) SAL-SAL ($n = 8$), (2) SAL-LPS ($n = 8$), (3) LPS-SAL ($n = 8$), and (4) LPS-LPS ($n = 8$). The statistical model included the random effect of cow, the fixed effects of period, square, treatment, and their interactions. Compared with SAL, LPS increased vaginal temperature from 2 to 6 h post-infusion by 0.9°C (maximum 39.7°C at 4 h). The LPS increased heart rate (89.4 vs. 74.5 ± 5.2 bpm) at 6 h post-infusion and respiratory rate within the first 2 h (45.2 vs. 32.5 ± 4.2 rpm). In P4, SAL-LPS induced a similar temperature increase (0.9°C , maximum 39.7°C at 4 h) compared with SAL-SAL and LPS-SAL. However, LPS-LPS induced a stronger febrile response than SAL-LPS (maximum 40.5°C vs. 39.7°C). These findings suggest that repeated endotoxin challenges potentiate metabolic and immune responses, potentially training the immune system and modulating adaptation to inflammatory stress in dairy cows.

Key Words: lipopolysaccharide (LPS), endotoxin tolerance, dairy cow

1103 Pooled analysis of blood BHB dynamics associated with health, production, and reproduction in dairy cows. T. C. Bruinje^{1,2}, R. B. Paiano², and S. J. LeBlanc², ¹Dairy and Food Science, South Dakota State University, Brookings, SD, ²Population Medicine, Guelph, ON, Canada.

Our objective was to investigate the association of dynamics of blood BHB measured at wk 1 (BHB1) and wk 2 (BHB2) postpartum with a single outcome combining health, production, and reproduction. A pooled data analysis with records from 9 studies, 57 herds, and 7,371 cows was conducted. The BHB1 and BHB2, calving BCS (wk -3 to 1), and calving-related disorders (CalvD; twins, dystocia, RP, milk fever) were assessed. Cows were classified as being successful (22% of cows) if they had all of (1) no subsequent clinical disease, (2) first test fat- and protein-corrected milk yield above herd- and parity-specific medians, and (3) pregnancy within 60 d after the voluntary waiting period. Logistic regression models were stratified by parity, BCS, or CalvD, and included the fixed effects of BHB1 and BHB2 (linear and quadratic terms), the covariates not included in the strata (parity, BCS, or CalvD), and farm nested within study as random. A spline curve was fitted to identify the inflection points (InfP) of BHB in quadratic associations with the predicted probability of being successful (PPS). All significant associations of BHB and PPS were negative, presented in Table 1. The quadratic association of BHB indicated that PPS increased up to the InfP, then declined with increasing BHB. Such associations were observed for BHB1 in parity 1, no CalvD, or BCS ≥ 3.75 strata, and for BHB2 in most strata. For multiparous cows, however, BHB2 InfP were high (2.0–2.2 mM). Blood BHB1 and BHB2 can be positively associated with performance up to a certain BHB level, beyond which performance declines with greater BHB. However, this relationship varied according to parity, CalvD, and BCS. Strategies to monitor and manage hyperketonemia could be refined to specific subgroups of cows.

Key Words: hyperketonemia, ketosis, transition health

1104 Differences in liver metabolism, inflammation, and behavior associated with vaginal discharge characteristics in peripartum dairy cows. F. N. S. Pereira¹, A. Martelo-Pereira¹, M. H. Rashid¹, E. F. Santos¹, M. N. Potter¹, C. S. McConnel¹, J. R. Wenz¹, H. F. Monteiro², J. Ranches³, R. C. Neves⁴, E. Trevisi⁵, and C. C. Figueiredo¹, ¹Washington State University, Pullman, WA, ²University of California, Davis, CA, ³Oregon State University, Burns, OR, ⁴Purdue University,

West Lafayette, IN, ⁵Università Cattolica del Sacro Cuore, Piacenza, Italy.

Our objectives were to assess liver metabolism, uterine inflammation, and behavior associated with different postpartum vaginal discharge (VD) characteristics in Holstein dairy cows (n = 244). Vaginal discharge was visually assessed at 4, 7, and 10 DIM on a Washington dairy. Cows were grouped by the highest VD score as follows: VD3 (n = 82): clear or mucopurulent discharge with $<50\%$ pus; VD4 (n = 97): mucopurulent discharge with $\geq 50\%$ pus or non-fetid watery reddish-brown discharge; and VD5 (n = 65): fetid, watery, reddish-brown discharge. Blood was collected at d -14, -7, 1, 3, and 7 relative to calving, and albumin, cholesterol, and bilirubin concentrations measured to calculate the liver health index (LHI; positive values associated with improved cow performance/health). Daily rumination, activity, and health index (activity monitor) averages from d 14 before parturition to 14 DIM were analyzed by period (pre- and postpartum). Uterine cytology performed at 7 DIM was used to assess the prevalence of polymorphonuclear leukocytes (PMN) in the uterus. Analysis of variance models with repeated measures considering cow nested within VD group as random effect were used. Prepartum behavior varied by group ($P < 0.05$); VD5 showed lower daily rumination and activity time (469 and 445 min) compared with VD3 (502 and 458 min) and VD4 (488 and 446 min). Postpartum behavior differed by group ($P < 0.01$), VD5 showed significantly lower daily rumination and activity time (490 and 448 min, respectively) compared with VD3 (525 and 462) and VD4 (515 and 455). Although prepartum health index was similar, VD5 had a smaller index ($P < 0.01$; 96.6) than VD3 (99.3) and VD4 (98.9) postpartum. No differences in LHI were observed among groups prepartum; however, VD5 postpartum LHI (-0.64) was smaller compared with VD3 (0.51) and VD4 (0.08). Postpartum uterine inflammation tended to differ ($P = 0.06$) among groups. A greater prevalence of PMN was observed between VD5 and VD3 (49.0% vs. 32.7%) but not between VD4 (40.3%) compared with VD3 or VD5. In conclusion, VD5 was associated with differences in behavior, liver metabolism, and a greater degree of uterine inflammation.

Key Words: uterus, metabolism, liver

1105 The role of prepartum inflammatory status in transitioning dairy cows' adaptation. G. Meli^{1,2}, G. G. Begalli¹, L. D. P. Maldonado^{1,3}, V. A. de Oliveira^{1,4}, A. F. Lima¹, J. Halfen¹, E. Trevisi⁵, T. Fernandes¹, and J. S. Osorio¹, ¹School of Animal Sciences, Virginia Tech University, Blacksburg, VA, ²Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, Lodi, Italy, ³Universidad de La Salle, Bogotá, DC, Colombia, ⁴Universidade Federal de Lavras, Lavras, MG, Brazil, ⁵Department of Animal Sciences, Food and Nutrition (DIANA), Faculty of Agriculture, Food and Environmental Sciences, Università Cattolica del Sacro Cuore, Piacenza, Italy.

Inflammation in transition dairy cows remains a puzzling phenomenon. While high inflammation may lead to an overstimulated immune system, low inflammation may render cows at risk of infectious diseases postpartum. The objective was to evaluate the effects of different prepartal planes of inflammation on feed intake, lactation performance, and immunometabolism. Thirty-nine multiparous Holstein cows were housed in a freestall barn and followed from -35 to 60 DIM. Cows were retrospectively classified into 3 groups based on serum haptoglobin (Hap) concentration at -7 DIM: HP1 (n = 13; Hap = 0.46 g/L), HP2 (n = 13; 0.16 g/L), and HP3 (n = 13; 0.09 g/L). Blood samples were collected at -35, -28, -14, -7, 3, 7, 14, and 21 DIM to evaluate biomarkers related to metabolism, oxidative stress, minerals, inflammation, and liver function. Statistical analysis was performed using the MIXED procedure of

Table 1 (Abstr. 1103). Associations of BHB1 and BHB2, and respective InfP, with PPS

Strata	BHB1		BHB2	
	Effect ¹	InfP	Effect ¹	InfP
Parity				
1	Quad.	1.3	Quad.	1.5
2	NS	.	Quad.	2.0
≥ 3	Linear	.	Quad.	2.2
CalvD				
No	Quad.	1.1	Quad.	1.6
Yes	Linear	.	Quad.	1.4
BCS				
≤ 3.25	NS	.	NS	.
3.50	Linear	.	Quad.	1.2
≥ 3.75	Quad.	0.9	Quad.	1.3

¹Associations were linear, quadratic (Quad.), or non-significant (NS).

SAS. Significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$. After calving, dry matter intake (DMI; $P = 0.06$) tended to be greater in the HP2 group compared with HP1 and HP3 (24.6 vs. 22.5 and 22.7 kg/d). Similarly, milk yield tended ($P = 0.09$) to be greater in HP2 compared with HP1 and HP3 (50.5 vs. 48 and 45.6 kg/d). As expected, a group effect ($P = 0.01$) on Hap was observed, where HP1 (0.25 ± 0.13 g/L) had greater ($P < 0.01$) Hap than HP3 (0.16 ± 0.13 g/L), whereas Hap in HP2 (0.20 ± 0.14 g/L) was similar ($P \geq 0.11$) to HP1 and HP3. At -7 DIM, HP1 had greater ($P \leq 0.03$) blood myeloperoxidase (MPO) than HP2 and HP3 (450.57 ± 19.61 vs. 391.53 ± 19.61 and 38074 ± 19.61 U/L), confirming a greater systemic inflammation status in HP1 cows. Lower ($P \leq 0.02$) blood Mg was observed in HP1 compared with HP2 and HP3, while HP2 and HP3 had similar ($P = 0.3$) Mg levels (0.94 ± 0.01 vs. 0.98 ± 0.01 and 1.00 ± 0.01 mmol/L). Overall glutamic-oxaloacetic transaminase was lower ($P < 0.01$) in HP3 compared with HP2 (92.61 ± 1.04 vs. 108.76 ± 1.04 U/L). Prepartal plane of inflammation did not affect blood Ca ($P = 0.86$), NEFA ($P = 0.70$), or reactive oxygen metabolites (ROM; $P = 0.45$). Intermediate prepartal haptoglobin levels seemed to positively influenced feed intake and lactation performance, underscoring the need for characterizing an adequate inflammatory state before calving to support the animal's adaptation during the transition period.

Key Words: inflammation, transition period, biomarker

1106 Association between early postpartum haptoglobin concentrations and early lactation maladaptation in multiparous Holsteins. J. A. Seminara* and J. A. A. McArt, *Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY.*

Dyscalcemia (total serum calcium ≤ 2.2 mmol/L at 4 DIM) is a useful marker of early lactation maladaptation, and dyscalcemic cows have elevated blood concentrations of haptoglobin, an inflammatory marker, as early as 2 DIM. Therefore, our objective in this exploratory prospective cohort study was to establish whether haptoglobin concentration at 2 DIM is associated with maladaptation in multiparous Holstein cows. We hypothesized that greater haptoglobin concentrations would be associated with increased risk of dyscalcemia at 4 DIM, decreased milk yield, and increased risk of herd removal within 30 DIM. To test these hypotheses, we collected blood from 267 multiparous Holsteins on 4 farms in New York at 2 DIM for haptoglobin assessment and at 4 DIM for dyscalcemia diagnosis, as well as records on milk production through 10 wk of lactation and herd removal through 30 DIM. We divided cows into quartiles based on blood haptoglobin concentrations at 2 DIM (1st quartile: LO; 2nd quartile: MED-LO; 3rd quartile: MED-HI; 4th quartile: HI) and compared outcomes among the 4 groups using Poisson regressions for dyscalcemia and herd removal and linear mixed effects models with a random effect of cow for milk yields. All models included parity group and farm. Compared with the LO group, risks of dyscalcemia were increased for each quartile whereby the risk ratio (RR $\pm$ 95% CI) for MED-LO cows was 3.0 (0.9 to 9.4; $P = 0.07$), for MED-HI cows was 4.3 (1.4 to 13.0; $P = 0.01$) and for HI cows was 10.9 (3.8 to 31.2; $P < 0.001$). We found that LO, MED-LO, and MED-HI cows did not differ in milk yields over 10 wk in milk; however, HI cows produced 4.5 to 9 kg less milk per d than all other groups through 8 wk in milk (all adjusted $P < 0.05$). With respect to herd removal within 30 DIM, cows in the HI group had a RR of 6.0 (0.7 to 54.8; $P = 0.1$) when compared with the LO group. Overall, our results suggest that blood haptoglobin concentrations at 2 DIM could be a useful early marker of lactational maladaptation, hinting at underlying mechanisms that may be driving suboptimal performance.

Key Words: inflammation, dyscalcemia, milk yield

1107 Relationships between systemic inflammation, subclinical hypocalcemia, and hyperketonemia in clinically healthy Holstein cows. N. Taechachokevivat*, L. Avila-Granados, J. Grantz, A. Ueda, and R. Neves, *Purdue University, West Lafayette, IN.*

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

The objectives of this study were to (1) assess associations of plasma haptoglobin (Hp) at 1 and 3 DIM with subclinical hypocalcemia (SCH) and hyperketonemia (HYK) in clinically healthy cows and (2) assess associations of SCH, HYK, and Hp with milk yield. Blood samples were collected from 513 cows from 2 herds at 1, 2, 3, 5, and 7 DIM, and 428 clinically healthy cows remained for the final analyses. Plasma Hp, total Ca, and blood β -hydroxybutyrate (BHB) were analyzed. Multivariable Poisson regression models were used to assess associations of Hp at 1 DIM with risks of SCH in primiparous cows (total Ca ≤ 2.15 mmol/L at 2 DIM) and Hp at 1 and 3 DIM with SCH in multiparous cows (total Ca ≤ 2.20 mmol/L at 3 and/or 5 DIM) and HYK (BHB ≥ 1.2 mmol/L at 3, 5, and/or 7 DIM). Critical Hp thresholds were derived from receiver operating characteristic curves. For objective 2, cows were categorized into 4 groups: (1) NON; low Hp on all DIM, no SCH or HYK ($n = 223$), (2) MET; low Hp on all DIM, with SCH and/or HYK ($n = 35$), (3) INF; high Hp on ≥ 1 d, no SCH or HYK ($n = 122$), and (4) INF+MET; high Hp on ≥ 1 d, with SCH and/or HYK ($n = 48$). Thresholds for Hp were based on a previous study from our group (primiparous: 0.4, 0.4, and 0.6 g/L at 3, 5, and 7 DIM, respectively; multiparous: 0.2, 0.3, 0.5, and 0.1 g/L at 1, 3, 5, and 7 DIM, respectively). Linear mixed models were used to assess associations of inflammatory-metabolic status with milk yield up to wk 12 of lactation. Multiparous cows with Hp > 0.5 g/L at 3 DIM were 5.6 times more likely to have SCH (CI 2.8–11.3, $P = 0.002$). Cows with Hp > 0.495 g/L at 3 DIM were 3.5 times more likely to have HYK (CI 2.0–6.0, $P = 0.0009$). Primiparous INF+MET cows produced more than INF and NON cows with a group $\times$ wk interaction. Multiparous INF and INF+MET cows produced less than NON and MET cows in the first 2 mo. These results suggest that systemic inflammation is associated with SCH, HYK, and reduced milk yield in clinically healthy cows. Concurrent metabolic disease with inflammation decreased production in multiparous cows but increased it in primiparous cows.

Key Words: systemic inflammation, subclinical hypocalcemia, hyperketonemia

1108 Associations of plasma Ca with cow performance at the intersection of disease in early postpartum. A. Fraz*, M. B. Poindexter, J. E. P. Santos, and C. D. Nelson, *University of Florida, Gainesville, FL.*

We hypothesized that the associations between delayed hypocalcemia and cow performance depended on diagnosis of postpartum disease. A retrospective cohort study was conducted using plasma calcium (Ca), disease, and performance data from 1,094 multiparous cows. The study objectives were to test the associations of plasma Ca at d 1 and 3 postpartum with milk yield up to 10 wk, pregnancy per AI at d 32 and 60 after first insemination, and blood markers, both in the absence and presence of disease. Morbidity affected 24% of the cows in the first 7 d postpartum (retained placenta, metritis, mastitis, lameness, digestive disorders, milk fever, and death). Cows were classified based on plasma Ca ≤ 1.77 mM or > 1.77 mM at 1 d postpartum and again based on plasma Ca ≤ 2.20 mM or > 2.20 mM at 3 d postpartum. Data analyses were performed separately for classifications of Ca at d 1 and 3 postpartum. Models included the fixed effects of morbidity, plasma Ca category, and their interaction. Additional covariates such as lactation number,

Table 1 (Abstr. 1108). Associations between plasma Ca at d 1 and 3 postpartum and cow performance, based on morbidity

Item	Non-morbid		Morbid		SEM	P-value		
						Morbidity	Ca	Morbidity × Ca
Day 1 Ca, mM	≤1.77	>1.77	≤1.77	>1.77				
Milk yield, kg/d	55.8	54.8	52.2	52.0	0.5	<0.001	0.16	0.30
Haptoglobin, ¹ µg/mL log ₁₀	2.26	2.17	2.68	2.64	0.04	<0.001	0.04	0.41
BHB, ¹ mM	0.70	0.67	0.79	0.71	0.02	<0.001	0.008	0.18
Pregnancy/AI d 32, %	42.2	45.8	47.1	38.5	9.8	0.72	0.53	0.14
Pregnancy/AI d 60, %	40.8	41.6	39.3	35.1	10.7	0.33	0.66	0.53
Day 3 Ca, mM	≤2.20	>2.20	≤2.20	>2.20				
Milk yield, kg/d	54.9 ^a	54.8 ^a	50.1 ^c	52.7 ^b	0.5	<0.001	0.02	0.008
Haptoglobin, ¹ µg/mL log ₁₀	2.25	2.15	2.71	2.55	0.04	<0.001	<0.001	0.36
BHB, ¹ mM	0.69 ^b	0.67 ^b	0.80 ^a	0.64 ^b	0.02	0.03	<0.001	<0.001
Pregnancy/AI d 32, %	47.9	47.5	38.3	52.1	8.6	0.47	0.10	0.09
Pregnancy/AI d 60, %	43.1 ^a	40.6 ^{ab}	31.2 ^b	46.0 ^a	10.2	0.32	0.10	0.03

¹Measured in plasma at 3 DIM.

calving problem, BCS at calving, calving season, and gestation length were included in the models if $P < 0.10$. Results are in Table 1. The poor cow performance associated with hypocalcemia at d 3 postpartum is likely due to the presence of disease.

Key Words: calcium, inflammation, morbidity

1109 Inflammation does not impact calcium supplementation efficacy in early-lactation Holstein cows. J. M. Poggi*, J. A. Sem-inara, and J. A. A. McArt, *Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY.*

Dyscalcemia and systemic inflammation in early-lactation dairy cows increase disease risk, reduce feed intake, and impair milk production and reproduction. This study evaluated whether calcium supplementation improves milk yield in cows with inflammation-associated dycalcemia, measured by elevated haptoglobin (HP) and serum amyloid A (SAA) at 2 DIM. Serum samples collected at 2 DIM from a trial where cows were randomized to a no-treatment control ($n = 267$) or delayed administration of a 43 g oral calcium bolus at 2 and 3 DIM ($n = 270$) were analyzed. Cows came from 4 farms, and both treatments were administered across all 4 farms. Using serum results, cows were categorized into quartiles based on HP or SAA concentrations. Linear mixed models, adjusting for farm, parity, and random cow effects, assessed the impact of calcium supplementation between quartile groups on milk yield over time. Quartile groups were $HP \leq 0.32$ mmol/L (LO_{HP}), $0.32 < HP \leq 0.51$ mmol/L ($MED-LO_{HP}$), $0.51 < HP \leq 0.88$ mmol/L ($MED-HI_{HP}$), and $HP > 0.88$ mmol/L (HI_{HP}), with SAA grouped as $SAA \leq 8.2$ mg/L (LO_{SAA}), $8.2 < SAA \leq 14.3$ mg/L ($MED-LO_{SAA}$), $14.3 < SAA \leq 21.4$ g/L ($MED-HI_{SAA}$), and $SAA > 21.4$ mg/L (HI_{SAA}). Calcium supplementation at 2 DIM did not affect milk production compared with non-supplemented controls in any HP quartile (HI_{HP} , control = 45.0 [43.0, 46.9] kg/d, bolus = 45.5 [43.9, 47.1] kg/d; $MED-HI_{HP}$, control = 48.4 [46.6, 50.1] kg/d, bolus = 49.5 [47.7, 51.3] kg/d; $MED-LO_{HP}$, control = 48.9 [47.2, 50.7] kg/d, bolus = 48.8 [47.2, 50.5] kg/d; LO_{HP} , control = 50.0 [48.4, 51.7] kg/d, bolus = 49.6 [47.6, 51.6] kg/d; $P = 0.81$). Similarly, we found no difference in milk production between calcium supplementation groups based on SAA (HI_{SAA} , control = 46.4 [44.7, 48.1] kg/d, bolus = 46.2 [44.4, 48.0] kg/d; $MED-HI_{SAA}$, control = 47.9 [46.1, 49.7] kg/d, bolus = 47.9

[46.1, 49.7] kg/d; $MED-LO_{SAA}$, control = 49.3 [47.4, 51.1] kg/d, bolus = 48.5 [46.8, 50.2] kg/d; LO_{SAA} , control = 49.3 [47.5, 51.2] kg/d, bolus = 49.3 [47.6, 51.1] kg/d; $P = 0.97$). Our findings suggest oral calcium supplementation at 2 DIM does not mitigate inflammation-associated reductions in milk yield, when postpartum inflammation is assessed.

Key Words: inflammation, serum amyloid A, haptoglobin

1110 Effects of systemic inflammation on performance, metabolism, and immune responses during an intramammary *Streptococcus uberis* challenge in postpartum dairy cattle. A. Celemin Sarmiento*, M. Bulnes-Larsen¹, C. Chase², and T. H. Swartz¹, ¹Department of Dairy and Food Science, South Dakota State University, Brookings, SD, ²Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD.

Low-grade systemic inflammation has been associated with postpartum diseases including mastitis in dairy cattle. Nevertheless, these associations do not demonstrate a cause-and-effect relationship. Therefore, the objective of this study was to evaluate the effects of systemic inflammation in postpartum dairy cattle on performance, metabolism, and immune responses during an intramammary challenge. Parous Holstein dry cows were blocked by parity, expected calving date, and genetic merit for milk yield (PTAM) and randomly assigned within block to receive a daily subcutaneous injection of either (1) recombinant bovine tumor necrosis factor α (rbTNF α , $n = 10$; 2 µg/kg BW in 10 mL of PBS) or (2) saline (CON, $n = 10$; 10 mL of PBS) from d 1 to 7 postpartum. All cows received an intramammary *Streptococcus uberis* challenge (2,500 cfu) in a randomly selected rear quarter at 4 d postpartum. Milk (composite and quarter-level) and blood samples were collected daily. Data were analyzed using linear mixed models (PROC GLIMMIX in SAS) including the fixed effects of treatment, time, and the interaction. Cows treated with rbTNF α produced less milk (28.2 vs. 32.9 ± 1.2 kg/d; $P = 0.01$), milk protein (1.1 vs. 1.3 ± 0.05 kg/d; $P < 0.01$), milk fat (1.4 vs. 1.5 ± 0.05 kg/d; $P = 0.02$), and energy-corrected milk yields (35.7 vs. 40.7 ± 1.1 kg/d; $P < 0.01$) than CON. Similarly, cows treated with rbTNF α tended to consume less dry matter than CON (16.5 vs. 20.0 kg/d; $P = 0.06$). Plasma free fatty acid concentrations were greater in rbTNF α than CON (525 vs. 437 ± 31 µmol/L, $P < 0.01$); however, no differences were found for glucose ($P = 0.80$) or β -hydroxybutyrate (P

= 0.52). Finally, no difference was found for *S. uberis* bacterial counts ($P = 0.51$), challenged quarter somatic cell score (SCS; $P = 0.98$), or composite SCS ($P = 0.32$) during the intramammary challenge. In conclusion, systemic inflammation induced by rbTNF α in postpartum

dairy cows reduced milk production, enhanced lipolysis, but did not affect mammary gland immune responses during a *S. uberis* challenge.

Key Words: cytokine, mastitis, immunometabolism

Lactation Biology Symposium: How the Mammary Milieu Affects Udder and Neonatal Health: From Microbes to Antibodies

1255 Revisiting the potential of protective intramammary infections in dairy cattle. P. R. F. Adkins*, *Department of Veterinary Medicine and Surgery, University of Missouri, Columbia, MO.*

An intramammary infection (IMI) is defined as the presence of an infectious organism in the mammary gland. In general, IMI negatively impact both milk quality and quantity and therefore result in major economic loss to the dairy industry. The National Mastitis Council's mastitis control plan was developed to improve milk quality by reducing IMI associated with contagious and environmental organisms. While it is best practice to work to reduce the prevalence of all IMI, some minor mastitis pathogens have been found to have minimal negative impacts on both somatic cell count and milk yield. Furthermore, beyond a lack of negative effects, some minor mastitis pathogens may provide protection for the gland. For example, previous research has identified that when quarters were infected with minor pathogens, such as *Corynebacterium* species, infections with major pathogens were significantly lower than in comparable control quarters not infected with minor pathogens. Additionally, others have previously identified that when quarters were infected with *Staphylococcus chromogenes*, fewer quarters that were challenged by infusion of *Staphylococcus aureus* became infected compared with uninfected quarters that were similarly challenged with *S. aureus*. These protective effects may extend beyond the gland, as teat end colonization with *S. chromogenes* has been associated with a low somatic cell count in early lactation. Possible protective mechanisms of these organisms include bacteriocin production and competitive exclusion. Bacteriocins are bactericidal peptides produced by bacteria to inhibit the growth of similar or closely related bacterial species. Whole-genome sequencing has identified that approximately 20% of non-*aureus* staphylococcal species have the potential to produce bacteriocins. Further research working to elucidate the protective mechanisms provided by these bacterial species could result in novel mastitis control and prevention strategies.

Key Words: mastitis, bacteria, heifer

1256 Mastitis and the milk microbiome: Challenging old assumptions, embracing new insights. J. A. Ferronato*, *The Pennsylvania State University, University Park, PA.*

The study of microbial communities has advanced significantly through improved culture-independent molecular techniques, which have revealed the complex roles microorganisms play in both promoting health and causing disease. The bovine mammary gland has historically been considered sterile, with intramammary infections caused by entry of pathogens via the teat canal. This belief is supported by the effectiveness of mastitis control measures, aimed at preventing pathogen entry. But, recent microbiome studies challenge this paradigm, suggesting that the udder may harbor its own microbiota, potentially transforming mastitis into a condition associated with dysbiosis rather than infection. Despite this evidence, the assertion of a stable and functional mammary microbiota is complicated by the inherent risk of contamination in low-biomass samples; minimal external DNA can significantly distort microbiome analyses. Also, the absence of standardized protocols hinders data reproducibility and comparability. Furthermore, there is currently no strong evidence for the existence of an entero-mammary pathway in cows, a mechanism that facilitates immune cell migration and microbial translocation from the gut to the mammary gland in other

species. Nonetheless, it has been proposed that the mammary gland could become colonized at or around the time of the first lactation; opening of the teat canal could allow bacterial translocation from environmental or skin-associated microbiota. Thus, early microbial colonization might influence mastitis incidence throughout a cow's productive life. However, there is currently no strong evidence that calves are born with a pre-established mammary microbiota, as seen in other mucosal sites. If a mammary microbiota is proven to exist, it would prompt reevaluation of mastitis prevention strategies, which have focused on lactating cows, to consider the role of early-life colonization and its long-term effects on udder health. In conclusion, while the hypothesis of a bovine mammary microbiota remains controversial, emerging molecular evidence and new approaches to mastitis control present an exciting opportunity for further research.

Key Words: mammary microbiome, mastitis

1257 Mucosal immunity and antibody trafficking in the dairy cow mammary gland. S. Langel*, *Center for Global Health and Diseases, Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, OH.*

Antibodies are vital components of the adaptive immune system and are transferred passively from mother to offspring via breast milk across all mammalian species, providing critical protection during early life. Milk contains multiple immunoglobulin isotypes—including IgG, IgA, and IgM—in species-specific proportions. Among these, IgA predominates at mucosal surfaces and plays a key role in targeting both pathogenic microbes and commensal organisms in the neonatal gut. Evidence from several species, including rodents and swine, indicates that IgA-secreting plasma cells can migrate from the intestinal mucosa to the lactating mammary gland via the gut–mammary axis, facilitating the secretion of mucosal IgA into milk. Recent findings from our group also support the existence of an intranasal–mammary axis, in which IgA-secreting cells originate in the nasal mucosa traffic to the mammary gland and contribute to the IgA pool in colostrum and milk. In this talk, I will explore the evidence for the gut–mammary antibody axis in dairy cattle and compare it to what is known in other species. I will also discuss its implications for protecting against intramammary infections in heifers and cows, as well as enteric pathogens in calves. Understanding the origins of antibodies in bovine colostrum and milk will be critical for developing therapeutic strategies aimed at boosting protective antibody levels before calving, with the goal of enhancing disease resistance in both the dam and her offspring.

Key Words: immunoglobulin, mastitis, colostrum

1258 Transmission of gut virome in cow/environment-to-calf model and its regulatory influence on the microbiome. F. X. Zhao*, S. Liu, T. Y. Chen, Y. M. Zhuang, M. Ma, and Z. J. Cao, *State Key Laboratory of Animal Nutrition and Feeding, International Calf and Heifer Organization, College of Animal Science and Technology, China Agricultural University, Beijing, China.*

This study investigates the composition of the cow gut virome before and after parturition, its transmission to offspring, and its impact on the gut microbiome of dairy calves. Samples were collected from 37

cows (rumen fluid, feces) at 2 critical time points (21 d pre-parturition and 0 d post-calving), paired calf meconium within 30 min of birth (pre-colostrum and separated from his mother), and environmental samples (wastewater, soil). A total of 177 metagenomes and 122 meta-transcriptomes were sequenced, assembled via SPAdes/rnaSPAdes, and annotated using IMG and RVMT databases, revealing microbial taxonomy, metabolism, and active functions. Our findings reveal that DNA virome diversity is higher in cow rumen fluid, feces, and the calf oral cavity than in the calf gut. Post-parturition cow rumen fluid viromes more closely resemble fetal feces than pre-parturition samples. Fetal feces are enriched with eukaryotic viruses such as bovine polyomavirus and phages, whereas the calf oral cavity hosts a greater abundance of phages. The RNA virome in fetal feces exhibits higher diversity compared with post-parturition cow rumen fluid. Notably, the RNA viromes of wastewater and fetal feces share high similarity. SourceTracker and

genome alignment analyses show that the calf oral virome derives from post-parturition cow rumen fluid and soil, while the calf fecal virome is predominantly sourced from the oral cavity. Phages from *Actinomyces* and *Proteobacteria* in the oral cavity are also found in feces. Metagenomic analysis reveals a higher proportion of lytic phages in fetal feces and the calf oral cavity, increased phage-host interactions in cow rumen fluid, and distinct phage-host correlations between calves and cows. Many phage genomes contain auxiliary metabolic genes, including amino acid metabolism, cofactor metabolism, energy metabolism, and carbohydrate metabolism. This study provides valuable insights into the gut virome dynamics and its transmission from cows to calves, offering potential avenues for improving calf health management.

Key Words: dairy calf, virome transmission, phage-host dynamics

Breeding and Genetics Symposium: Joint ADSA-Interbull Session— Management of Genetic Diversity for Future-Proofing Dairy Cattle Breeding

1111 Identifying genetic diversity within indigenous and highly commercialized breeds for improved performance and future preservation. H. J. Huson*, *Cornell University, Ithaca, NY.*

Natural and artificial selection has diversified cattle into existing breeds and populations that are uniquely adapted to a variety of environments and specialized for different production measures. Genetic diversity provides the foundation for this phenotypic plasticity within cattle and is important for their long-term sustainability. Simply stated, genetic diversity is the biological variation within a species resulting from genetic variation. Yet diversity within dairy cattle is much more complex than calculating allele frequencies. Breeds such as the Holstein, Jersey, Brown Swiss, Ayrshire, and Guernsey have experienced intense selection pressure to be recognized as top producing dairy breeds. That selection pressure has increased levels of inbreeding and reduced genetic diversity within each breed, yet each breed now possesses unique, conserved haplotypes associated with their productivity that the industry capitalizes on. Therefore, in these highly commercialized breeds, genetic diversity must be maintained to mitigate inbreeding depression while also conserving the breed-specific haplotypes that make them renowned dairy cattle. In contrast, less restricted breeding of indigenous cattle populations increases their genetic diversity, making them more adaptable to changing environmental conditions, yet their worth for production is often undervalued due to limited selection for production. The demands of changing environments including climate, disease, and management practices, necessitate the preservation and informed use of genetic diversity in dairy cattle. This means that highly selected regions of the genome within commercialized breeds as well as broad diversity across breeds, especially that of indigenous populations which are locally adapted to a wide variety of environmental variables, should all be preserved. As geneticists, it is our responsibility to identify genetic diversity to inform breeding practices and germplasm preservation. We need to adapt and improvise our methods to look beyond the simple and see the value of complexity to best inform management practices.

Key Words: genetic diversity, indigenous, breed-specific haplotype

1112 Genetic resilience and the dynamics of inbreeding and diversity in dairy breeding. C. Maltecca*, F. Tiezzi², J. B. Cole³, J. Jiang¹, L. Ma⁴, and C. F. Baes⁵, ¹*Department of Animal Science, North Carolina State University, Raleigh, NC,* ²*Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, Florence, Italy,* ³*Council on Dairy Cattle Breeding, Bowie, MD,* ⁴*Department of Animal and Avian Sciences, University of Maryland, College Park, MD,* ⁵*Centre for Genetic Improvement of Livestock Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

Dairy breeding programs have been remarkably successful in increasing production levels over the past decades, whereby the focus is now increasingly shifting toward improving health, efficiency, product quality, and fertility. Given the nature of dairy selection, mating related animals is inevitable, and while the significant genetic improvements achieved through intense directional selection have so far outweighed any possible negative effects of increased homozygosity, the long-term consequences of this selection strategy remain uncertain. This includes challenges related to managing inbreeding and ensuring the overall sustainability of consistent selective pressure. In dairy populations,

there is a fragile balance between the accumulation of homozygosity, the elimination of harmful genetic variants, and the introduction of new genetic variation. Traditional breeding practices, which typically involved longer generation intervals, may have historically helped limit the spread of harmful variants and preserve genetic diversity. However, the adoption of genomic selection, which significantly shortens breeding cycles, has the potential to disrupt this balance. This has in recent years raised concerns about increased inbreeding load and the possible spread of detrimental mutations. Less attention has been given to the unquantified risk of a reduced genetic resilience, the population's ability to adapt and thrive in changing environments. To effectively manage these risks, it is crucial to deepen our understanding of the molecular basis of inbreeding depression, to quantify the current existing capacity to purge harmful variants within breeding populations and to attain a more atomic understanding of the overall genomic diversity of the global dairy herd. In addition to improving how we characterize genetic variation; it is paramount that we develop a more comprehensive insight into the dynamics of genetic variation recruitment and conversion within the context of modern selection practices and under different environmental conditions.

Key Words: genetics, inbreeding, diversity

1113 Harnessing indigenous African breeds for sustainable dairy production: Opportunities for crossbreeding to accelerate genetic improvement. N. Mapholi*, L. T. Nesengani¹, M. G. G. Chagunda², J. E. O. Rege³, I. Houaga^{1,2}, O. Opoola², C. Banga^{1,4}, E. M. Ibeagha-Awemu⁵, R. Mrode⁶, and A. Djikeng^{1,2}, ¹*College of Agriculture and Environmental Sciences, UNISA Science Campus, Florida, Johannesburg, South Africa,* ²*Centre for Tropical Livestock Genetics and Health (CTLGH), Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, United Kingdom,* ³*Emerge Centre for Innovations—Africa, Nairobi, Kenya,* ⁴*Department of Animal Science, Botswana University of Agriculture and Natural Resources, Gaborone, Botswana,* ⁵*Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada,* ⁶*Animal and Veterinary Department, Scotland's Rural College, Edinburgh, United Kingdom.*

The African dairy sector has long been central to poverty alleviation, food, and nutritional security. While commercial dairy production in Africa is limited to a few countries, milk production from Africa's ruminant systems, mostly cattle, but also goat, sheep, and camel, is deeply embedded in livestock production systems in the continent. Across different countries in Africa, dairy contributes significantly to the agricultural GDP. For example, its contribution is 30% in Tanzania, 4.6% in South Africa, and 44% in Kenya. The different levels of contribution across countries are related to the variable farming structures and production practices across the continent, including the utilization of indigenous breeds. Within the high diversity of indigenous ruminant breeds in Africa, some cattle breeds (e.g., East African Zebu, Boran, Kenana, Butana, Ankole, Gudali, and White Fulani cattle) with potential as commercial dairy or dual-purpose animals have demonstrated promising milk production potential under harsh conditions, and are generally found in regions where the dairy industry makes large contributions to agricultural outputs. On the other hand, indigenous breeds such as the Nguni in Southern Africa have often been associated with low milk pro-

duction of approximately 5.5 kg/d. Despite their exceptional adaptability to the harsh local conditions, most indigenous African cattle breeds are generally characterized by low milk production potential. Consequently, efforts such as targeted selective breeding, cross breeding (e.g., formation of Mwapwa breed) with exotic breeds and, recently, the use of genomics have been made to improve milk production while leveraging on the adaptation attributes of indigenous breeds. Some of the genomic approaches attempted include the use of digital genomic tools to improve productivity and profitability in smallholder dairy systems, and these have achieved notable success in sub-Saharan Africa. Notwithstanding the general lack of performance and pedigree data for use in breeding programs, efforts have also been made to utilize genomic information to improve milk production in crossbred cattle in East Africa. Results from this endeavor reported the accuracy range of 0.28 to 0.41 suggesting that there is potential to achieve significant improvements in dairy production through the implementation of genomic selection of young bulls in crossbred small-holder cattle populations. Indigenous cattle breeds, therefore, hold the promise to sustainable dairy production on the African continent. Achievement of this goal would, however, need to be underpinned by investments in high level technologies such as genomics and large-scale recording of phenotypic data. This is where the African Animal Breeding Network will play an important role through its flagship pillars, multi-country genetic evaluation, and professional development. Closing the dairy productivity gap in Africa represents a significant driver toward ensuring resilient and climate smart livestock systems in Africa.

Key Words: African indigenous cattle breed, digital genomic tool, adaptation attribute

1114 Genomic- versus pedigree-based inbreeding: 2 sides of the same coin. N. P. P. Macciotta^{*1}, D. Lourenco³, D. Vicario², L. Degano², I. Misztal³, and A. Cesarani¹, ¹University of Sassari, Sassari, Italy, ²ANAPRI, Udine, Italy, ³University of Georgia, Athens, GA.

The management of inbreeding in dairy cattle population represents a key point for the conservation of genetic diversity and for the development of the future cow. The level of inbreeding of a livestock population depicts its genetic history and gives clues for managing its future. Inbreeding coefficients have been traditionally calculated using the pedigree (INB_PED). This is considered as the expected inbreeding, and it relies on accurate and depth pedigrees that are not always available. Genomic technologies allow for the calculation of different metrics that represent the genomic inbreeding (INB_GEN) i.e., the realized inbreeding. The comparison between the different measurements offers the opportunity to consider inbreeding from different perspectives: for example, the genomic inbreeding can be calculated either at whole genome or chromosome level. In this study INB_GEN and INB_PED are compared in different cattle breeds, either dairy or dual purpose, with

different levels of selection. The INB_GEN was calculated both from the genomic relationship matrix and from the runs of homozygosity (ROH). The temporality of inbreeding phenomena was investigated through the ROH lengths. Relationships between inbreeding and contribution of the animal to the genomic relationship matrix were also investigated.

Key Words: inbreeding, genomic, diversity

1115 Managing genetic diversity: Strategies for sustainable livestock improvement. C. F. Baes^{*1}, G. Condello¹, J. B. Cole², C. Maltecca³, R. E. Jahnel¹, F. Malchiodi^{1,4}, B. Mekanjuola¹, P. Stothard⁵, F. S. Schenkel¹, and F. Miglior^{1,6}, ¹Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, ²Council on Dairy Cattle Breeding, Bowie, MD, ³Department of Animal Science, North Carolina State University, Raleigh, NC, ⁴Semex Alliance, Guelph, ON, Canada, ⁵University of Alberta, Edmonton, AB, Canada, ⁶Lactanet, Guelph, ON, Canada.

Genomic selection has increased selection accuracy and reduced generation intervals, thereby greatly accelerating genetic improvement in dairy cattle. Simultaneously, an increase in the rate of homozygosity has been concomitantly observed. The much-debated concerns about inbreeding depression, reduced genetic variance, and the expression of harmful recessive alleles have further intensified given this rapid increase. Sustaining long-term genetic progress within breeding programs is critical to ensuring adaptability to changing environments, and maintaining animal health and fertility. Using existing genomic evaluation infrastructure, potential methods for identifying detrimental haplotypes can be investigated, mating programs that optimize genetic progress while minimizing inbreeding rates can be explored, and the integration of genomic diversity metrics into selection indices developed. The widespread use of genomically derived metrics, such as runs of homozygosity and effective population size estimates, can provide deeper insights into the accumulation of homozygosity and its phenotypic consequences. In the US and Canada, national genomic surveillance systems are being developed to track and manage genetic defects, ensuring sustainable breeding strategies. Furthermore, genetic diversity conservation efforts such as germplasm repositories and cross-breeding strategies may offer opportunities for safeguarding variation. Finally, genome editing, selective introgression of beneficial alleles, and other advances in precision breeding present additional opportunities to maintain diversity. Through the integration of genomic technologies, mating strategies, and conservation efforts, balanced livestock breeding programs can be developed. A proactive approach to the management of genetic diversity will improve long-term adaptability, economic viability, and environmental resilience in dairy cattle populations.

Key Words: homozygosity, genetic diversity, livestock improvement

ADSA-GSD Competition: Dairy Foods Oral Semifinalists

1116 Understanding factors influencing wear behavior, tackiness, and textural properties in relation to high-speed processing of Cheddar cheese. P. Choudhary*, M. Garcia, J. Bond, and P. Sharma, *Utah State University, Logan, UT.*

Cheese slicing is a common high-speed operation used for converting a block of cheese into retail formats. The cheese industry often faces the issues of food waste and financial losses because of poor slicing ability due to either sticking to rotating blades or crumbling nature. It is important to identify critical factors affecting slicing ability. Therefore, the objective of this study was to understand the impact of aging on mass loss and find its relationship with material properties potentially to be used for predicting slicing behavior of cheese. Ten commercial Cheddar cheese samples were subjected to composition and material properties analysis. Extent of proteolysis was performed with pH 4.6 soluble nitrogen analysis and reported as intact casein (IC). Wear behavior (penetration depth-PD, rate of wear, and mass loss) and tack properties (tack force-TF and tack energy-TE) were analyzed on an Anton Paar MCR302 Rheometer using Pin in Disk (PID/44) and parallel plate (PP50) geometries respectively on a 5-mm-thick slice of cheese. Texture profile analysis was conducted on 2 cm³ cubes with P75 probe on a TA.XT plus texture analyzer. All the analytical tests were performed in triplicate and at 5°C, which resembles with the commercial cheese slicing temperature. The pH range of the samples was found in the range of 5.2 to 5.6, which positively correlated with wear properties suggesting too high pH cause more material loss possibly due to higher insoluble calcium content. With increased moisture content, higher tack force, more mass loss was observed suggesting cheese becoming stickier. The IC to moisture ratio was found to be negatively correlated with tack force and tack energy, PD, rate of wear, and mass loss ($r > 0.8$), indicating that higher IC content can help with reduction in cheese sticking/adhesion to the blade and reducing mass loss during the high-speed operations (i.e., slicing). With increasing fat content (31.6% to 36%), hardness increased, and tackiness and PD decreased suggesting fat at low temperature (5°C) reinforces the cheese matrix (unlike room temperature) and favors slicing ability. Hardness, gumminess, and chewiness of cheese were negatively correlated ($r > 0.6$) tack properties indicating that the sample would be less sticky on the metal surface if it is harder or chewier, and therefore, suitable slicing. This research would help the cheese industry to develop strategies for reducing operational losses during high-speed slicing operations.

Key Words: wear behavior, tackiness, cheese slicing

1117 Impact of protein concentration on the cold gelling behavior of reconstituted micellar casein dispersions. J. Shekin J* and P. Sharma, *Utah State University, Logan, UT.*

Cold gel formation in ready-to-mix high protein beverages upon storage poses a significant quality challenge. The formation of gels at low temperatures in highly concentrated micellar casein concentrate (HC-MCC) can be attributed to the balance of casein-casein and casein water interactions in the presence of ionic calcium. This study investigates the rheological changes during cold gel formation with varying protein concentration of reconstituted micellar casein concentrates. Cold gelling temperature (CGT) and gel strength (G') across 8 protein concentrations ranging from 5% to 23% during cooling were tested in triplicate using an Anton-Paar MCR 302 rheometer. The effect of protein concentration of various physicochemical properties was tested at 5% level of

significance. The experimental protocol involved 3 stages: holding the dispersion at 60°C for 1 h, ramping the temperature down from 60°C to 5°C at 1°C per minute, and curing the gel by holding at 5°C for 12 h. The viscoelastic properties were continuously monitored throughout the experimental process. Change in the particle size distribution upon cooling from 60°C to 5°C was analyzed using the Anton-Paar Litesizer 500. Cold gel formation was not observed below a 15% protein concentration. As the concentration increased from 15% to 20%, the CGT increased from 8.9°C to 35°C, accompanied by a 35-fold increase in gel strength (G'). At 23% protein concentration, the dispersion exhibited gel-like characteristics even at 60°C, showing that increasing protein concentration alone can significantly elevate the gelling point. A sharp increase in mean particle size from 100 to 180 nm was observed below 30°C, suggesting swelling of casein micelles at low temperature due to decreased hydrophobic interaction and increased protein-water interactions, thereby strengthening gel formation. Frequency dependence of storage modulus (G') decreased with increasing protein concentration, suggesting formation of a stronger network. During the curing stage, the storage modulus (G') doubled, indicating increased gel strength over time. These results highlight the critical role of protein concentration in cold gel formation and provide insights into optimizing the formulation of protein beverages to mitigate undesirable gelation.

Key Words: micellar casein concentrate, cold gel, rheology

1118 β -Lactoglobulin-enriched whey protein aggregates for high-acid ready-to-drink beverage application. Y. Lin* and H. Zheng, *North Carolina State University, Raleigh, NC.*

β -Lactoglobulin (β -lg), the primary protein in whey protein isolate (WPI), contains a free thiol group at Cys121 that can initiate thiol-disulfide exchange reactions among whey proteins in response to thermal treatment. Such reactions together with other intermolecular forces may induce the formation of protein aggregates that exist at a lower free energy state than their native forms. Therefore, it is reasonable to hypothesize that the β -lg enriched whey protein aggregates (β WPA) may have superior heat and structural stability. This study aimed to optimize the processing conditions for making β WPA from β -lg-enriched WPI suitable for high-acid ready-to-drink (HA-RTD) applications. This study employed a factorial experimental design with data analyzed using one-way ANOVA followed by Tukey's post hoc test to determine significant differences ($P < 0.05$). Whey protein dispersions (4% and 6% wt/wt) at pH 6.2 or 7.0 were subjected to batch heat treatment at 85°C for 5, 15, and 30 min to produce β WPA samples. Aggregates with a higher surface charge, a minimal visible phase separation, and a smaller mean particle size ($<1 \mu\text{m}$) were selected as candidate ingredients. The selected aggregate dispersions, β WPA1 (6% protein, pH 7, 85°C for 5 min) and β WPA2 (6% protein, pH 6.2, 85°C for 15 min) were adjusted to pH 3.5, diluted to 3% protein concentration and characterized for resulting model samples (HA- β WPA) underwent characterization, including particle size distribution (PSD), ζ -potential, surface hydrophobicity, Turbiscan Stability Index (TSI), and flow behavior. The results showed that prolonged heating does not necessarily improve particle colloid and phase stability. The selected HA- β WPA samples were then heat treated at 88°C for 2 min to obtain model HA-RTD1 and HA-RTD2, respectively. After heat treatment, the HA-RTD1 formed a paste-like texture, whereas the HA-RTD2 maintained a liquid form. Despite these textural differences, TSI measurements confirmed bulk phase stability for both HA- β WPA samples. Fluorescence analysis revealed higher surface hydrophobicity

in HA-βWPA1 as compared with HA-βWPA2 ($P < 0.05$). The higher surface hydrophobicity may enhance protein-protein interactions, which explains the paste-like texture observed in HA-RTD1. These findings highlight the importance of controlled heating conditions in tailoring βWPA structure and colloidal functionalities toward HA-RTD applications. This study provides scalable methods for manufacturing βWPA as ingredients with tunable functionalities for developing stable HA-RTD with different textural features.

Key Words: β-lactoglobulin-enriched whey protein isolate (WPI), high-acid ready-to-drink (RTD)

1119 Structural organization and physical properties of whey permeate powders. T. Y. B. Peng*, D. Sözeri Atik, and J. B. Ubbink, *University of Minnesota, St. Paul, MN.*

Liquid whey permeates, which are nutritionally valuable due to their high mineral and micronutrient content, are commonly converted into powders by a process involving concentration, crystallization and spray drying to facilitate handling, storage, and transportation. The resulting powders are however observed to be physically and chemically unstable, resulting in caking and browning during storage. To understand the cause of these instabilities, we studied the structure-property relationships of commercial whey permeate powders. Based on microscopy, X-ray diffraction, and differential scanning calorimetry, we proposed a 2-phase model of whey permeate particles, comprising 2 distinct particle morphologies: (1) particles with small lactose crystallites dispersed in a continuous amorphous phase, and (2) particles consisting of one or a few large lactose crystals that are bonded together by a thin, discontinuous amorphous surface layer. The amorphous phase of the permeate powders is characterized by a low glass transition temperature (T_g) that, for our commercial samples, varies between 7.6 ± 0.5 to $20.5 \pm 0.7^\circ\text{C}$ at water activity = 0.23, confirming the high instability. The dependence of the T_g of the whey permeate powders on the water content was modeled by the Gordon-Taylor equation. Result from one of the samples suggests that when the water content (associated with amorphous phase) is at 2% and 3% (wt/wt) w.b., the T_g of the whey permeate powder is 12.8 ± 0.5 and $-7.9 \pm 0.6^\circ\text{C}$, respectively. This confirms the high water sensitivity of whey permeate powders, as it is strongly plasticized by water. The amorphous fraction of the whey permeate powders was quantified by the change in heat capacity (DC_p) associated with the glass transition relative to the fully amorphous powder. Our results provide a mechanistic model that allows to analyze how structure, water relations, and physical properties of whey permeate powders impact the physical and chemical stability, providing insights for optimizing processing and storage, and laying the groundwork for further research on fundamental mechanisms of permeate powders caking and browning.

Key Words: glass transition, plasticization, water activity, phase behavior

1120 Influence of preaggregated whey proteins in physicochemical properties of high protein beverage systems. S. Roy* and J. K. Amamcharla, *University of Minnesota, Saint Paul, MN.*

The interaction between whey protein and casein in high protein systems affects their stability at high heat treatments such as ultra-high-temperature sterilization. The heat labile nature of whey proteins causes denaturation and aggregation reducing their applicability. This work aims to study the effects of preaggregated whey proteins on heat stability of high protein beverages. Two lots of acidified whey protein isolates (pH 3.5) were procured. After reconstituting to 8% protein (wt/

wt), divided into 2 parts followed by adjusting to pH 6.8 (UWPI) at 25°C in one part and another part was heated at 90°C for 10 min in a high shear mixer at 10,000 rpm and adjusted back to pH 6.8 and termed as treated (TWPI). The TWPI and UWPI were used as whey protein sources in model high protein systems containing 8 and 10% proteins with micellar casein in 80:20, 60:40, and 50:50 casein to whey protein ratios. The model systems were heated in an oil bath at 140°C for 15 s, the physicochemical properties were measured before and after heating in duplicates followed by 2-way ANOVA with 0.05 significance level. Viscosity of 8% protein samples did not change significantly after heating in different ratios of casein and whey protein regardless of the treatment of whey protein. At 10% protein level at a 50:50 ratio viscosity increased from 4.53 ± 0.08 to 11.96 ± 0.08 mPa·s for UWPI and from 8.57 ± 0.54 to 11.90 ± 2.21 mPa·s for TWPI. Particle size increased after heating in 60:40 and 50:50 ratios but the increase was higher in UWPI than TWPI samples in both protein levels. Particle size for 10% sample for 50:50 increased from 168.74 ± 2.93 to 284.22 ± 25.6 nm in UWPI but for TWPI samples it increased from 183.65 ± 4.44 nm to 243.35 ± 11.9 nm indicating the treatment of whey protein reduced the interaction with casein. Turbidity increased after heating in all samples with increasing whey protein concentrations in both treatments and UWPI samples showed higher turbidity than TWPI samples after heating. Therefore aggregating the whey protein at a lower pH can reduce interaction between casein and whey proteins and improve heat stability in high protein systems.

Key Words: whey protein, preaggregation, heat stability

1121 Effect of stabilizer type and concentration on the physical and rheological properties of ice cream. J. I. Agbawodike*, A. G. S. Soler, M. A. U. R. Alvi, and M. M. Monteagudo, *New Mexico State University, Las Cruces, NM.*

Careful selection of the number, type, and concentration of stabilizers is critical for achieving a desired structure and storage stability of ice cream. This study aims to evaluate the impact of type and concentration of stabilizers, individual and blend, on key properties of ice cream, including particle size, flow properties, hardness, meltdown, and shape retention. The tested stabilizers included a commercial blend (carrageenan, guar gum, locust bean gum, polysorbate 80, and mono- and diglycerides) and individual stabilizers (gum arabic, guar gum, and xanthan gum). The concentration of all stabilizers varied from 0.5 to 0% (wt/wt). Particle size distribution was unimodal for the blend, xanthan gum, and gum arabic, while 0.15% and 0.5% guar gum exhibited bimodal distribution. Gum arabic formulations had the most pronounced shear-thinning behavior, followed by blend, xanthan, and guar gum formulations. Samples formulated without stabilizers had the highest maximum meltdown ($62 \pm 4.74\%$) and exhibited an early onset of meltdown ($1,442 \pm 38.28$ s), while samples formulated with a 0.5% blend had the lowest maximum meltdown ($15 \pm 3.50\%$) and late onset ($3,326 \pm 240.70$ s) ($P < 0.05$). Additionally, samples formulated with a 0.5% blend retained $77 \pm 5.51\%$ of their shape after melting, followed by 0.5% guar gum ($68 \pm 5.34\%$), while 0.5% xanthan and 0.5% guar arabic had lower values ($52 \pm 4.89\%$ and $51 \pm 5.12\%$, respectively). Hardness values ranged from the lowest in samples formulated without stabilizer (25.13 ± 6.73 N) to the highest with 0.5% xanthan gum (267.27 ± 21.50 N) ($P < 0.05$). The 0.5% guar gum formulation exhibited the highest compliance values, while the 0.5% blend formulations had the lowest. Statistical analysis (ANOVA and Tukey HSD) confirmed significant differences among formulations for meltdown behavior, shape retention, and hardness. Nonlinear regression analysis was used to analyze the meltdown curves, flow curves, and creep-recovery. The outcome of

this investigation suggests that the meltdown behavior of ice cream can be controlled by carefully selecting stabilizers and their concentrations.

Key Words: ice cream, stabilizer, meltdown behavior

1122 Impact of UV-C irradiation at a 5-log microbial reduction dosage on native serum proteins of bovine milk. B. Barman*, H. Mohamed, J. W. Cusic, and D. Dallas, *Oregon State University, Corvallis, OR*.

Ensuring the microbial safety of bovine milk while preserving its nutritional integrity is a critical goal in dairy processing. Traditional heat-based pasteurization methods such as ultra-high temperature (UHT), high temperature, short time (HTST), and low temperature, long time (LTLT) are effective in reducing microbial loads but can degrade key bioactive serum proteins, potentially compromising their nutritional and functional properties. UV-C (UV-C) irradiation offers a promising non-thermal alternative, inactivating microorganisms by inducing pyrimidine dimers that disrupt bacterial DNA replication. In this study, 4 vegetative bacterial strains (*Escherichia coli* O157:H7, *Salmonella typhimurium*, *Listeria monocytogenes*, and *Staphylococcus aureus*) and 2 spore-forming strains (*Bacillus cereus*, *Bacillus subtilis*) were individually inoculated into 60-mL batches of bovine milk. The inoculated samples underwent UV-C treatment using a surface immersion lamp, targeting a 5-log reduction in both vegetative and spore-forming bacterial populations. Following treatment, lactoferrin and immunoglobulins (IgG, IgA, and IgM) in the milk serum were quantified using commercial enzyme-linked immunosorbent assay (ELISA) test kits. One-way ANOVA, followed by Tukey's post hoc test, was used to evaluate differences in the specific protein abundances among different treatment groups. Statistical significance was set at $P < 0.05$. The 5-log reduction dosages for *E. coli*, *S. typhimurium*, and *S. aureus* were 12 kJ/L, whereas *L. monocytogenes* was found to be the most UV-resistant vegetative bacteria, achieving similar reductions at 14 kJ/L (UV-1). Both the spore formers required a higher irradiation dosage of 40 kJ/L (UV-2) to achieve the same at 95% CI. The UV-1 produced protein retention levels statistically comparable to HTST for lactoferrin, IgG, and IgM, whereas UV-2 showed moderately lower retention. In contrast, UHT consistently led to near-complete protein degradation. Overall, these findings suggest that UV-C processing is a promising non-thermal alternative to conventional pasteurization methods for safeguarding both microbial safety and nutritional integrity of bovine milk.

Key Words: non-thermal processing, microbial reduction, ultraviolet-C irradiation, bovine milk

1123 Insights into the interaction of Aronia berry polyphenols with various milk proteins. T. Mao* and M. S. Mohan, *South Dakota State University, Brookings, SD*.

Limited evidence has been reported on the interaction of milk proteins with mixed polyphenols extracted from Aronia berries. Therefore, the study aims to elucidate the complex interactions of Aronia berry polyphenols with various milk proteins. The UV-vis absorption spectroscopy and fluorescence spectroscopy were used to examine the mechanisms of milk proteins (micellar casein, whey protein, and micellar casein and whey protein mixture in a 50:50 ratio) upon binding with Aronia berry polyphenols in a protein to polyphenols concentration ratio of 40:1, 20:1, and 10:1. The bound polyphenols were quantified by total phenolic content analysis through Folin Ciocalteu reagent and HPLC followed by a loading efficiency test. Our results observed that the interaction between milk proteins and Aronia berry polyphenols occurred

spontaneously, leading to the formation of complexes through a static quenching mechanism, with dynamic quenching also contributing to the interactions. Aronia berry polyphenols preferably bound to casein micelles ($42.88 \pm 4.70\%$) compared with whey proteins ($8.65 \pm 2.67\%$) in the solution with the highest protein to polyphenols concentration ratio of 40:1 ($P < 0.001$). Aronia berry polyphenols, especially cyanidin-3-galactoside and ferulic acid, showed a stronger binding affinity for caseins than for whey proteins, whereas 5-caffeoylquinic acid and 5-feruloylquinic acid preferentially bound to whey proteins rather than caseins. These findings elucidate the preferential binding of Aronia berry polyphenols to casein micelles compared with whey proteins, providing valuable insights into developing a tailored, cost-efficient polyphenol delivery system using milk proteins.

Key Words: polyphenol, whey protein, casein

1124 Consumer liking and willingness to pay for novel alcoholic upcycled yogurt acid whey beverages. D. G. Hauser*, L. M. Preston-Wilsey, C. Gerling, and S. D. Alcaine, *Cornell University, Ithaca, NY*.

Upcycling, or converting food processing waste into new food products, provides a major opportunity for the dairy industry to develop new whey-based products that deliver on consumer demand for sustainable, health-promoting beverages while mitigating the economic and environmental burdens of whey waste streams. The goal of this research was to assess consumer liking and willingness to pay (WTP) for beverages produced by fermenting hydrolyzed yogurt acid whey (YAW) or water and sucrose on grape pomace (GP, the skins and seeds leftover from winemaking). Four iterations of beverages were tested; water and sucrose (WA), a commercially available version of WA (CWA), a YAW beverage (WH), and a carbon filtered version of WH (WHCF). 113 consumers scaled their aroma, flavor, and overall liking of each beverage on a 9-point hedonic scale. After sensory assessment, participants were asked the maximum amount they would pay for each product they tested. Three types of WTP were captured: uninformed WTP after sensory assessment, WTP after being informed about upcycled/sustainable production processes, and WTP after being informed of the high vitamin and mineral content of the whey beverages. Mixed, zero-inflated hurdle regression modeling was used to study the effect of sustainability and micronutrient information on WTP after accounting for the effects of participant, product, product:participant interaction, and overall liking. In the zero-inflated model ($WTP = 0$ or 1), the odds of a positive WTP value increased 11.5-fold ($P = 0.0092$) when consumers had sustainability information for WH, and by 7.8-fold ($P = 0.0011$) for micronutrient information. For the conditional model ($WTP > 0$), sustainability information led to an increase in WTP of 8.9% ($P < 0.001$), 7.1% ($P = 0.026$), or 9.3% ($P = 0.013$) for CWA/WA, WH, and WHCF, respectively. Information on micronutrients led to an increase of 13.3% ($P < 0.001$) in WTP. These results provide strong insight into the motivators of purchase intent for sustainable and health-promoting beverages which can be leveraged by dairy and beverage industry stakeholders in developing novel product offerings.

Key Words: upcycling, yogurt acid whey, willingness to pay

1125 Implications of temperature fluctuations during frozen storage of low-moisture, part-skim mozzarella. D. Digvijay*^{1,2}, T. Huppertz^{2,3}, A. L. Kelly², and P. Lamichhane¹, ¹*Food Chemistry and Technology Department, Teagasc Food Research Center, Moorepark, Fermoy, Co. Cork, Ireland*, ²*School of Food and Nutritional Sciences, University College Cork, Cork, Ireland*, ³*FrieslandCampina, Amersfoort, the Netherlands*.

The short functional shelf-life of pasta-filata cheeses and their increasing global demand have attracted studies on the suitability of its frozen storage (FS). However, the role of temperature fluctuations (TF) during FS of cheeses (in logistics/retail) has not been studied. The present study (in triplicate trials) investigated the impact on rheological, microstructural, and water dynamics of industrial LMPS mozzarella when subjected to (1) rapid TF by applying repeated freeze-thawing cycles (FTC; 1–5 cycles; 6°C to –18°C to 6°C) using a blast freezer; and (2) slow TF (STF; at mild, moderate, and intense scales) using conventional chest freezer at –18°C. The results were compared against refrigerated cheese sample as control (CTR). Freezing and FS led to a significant ($P < 0.05$) increase in G' and G'' values at 25°C compared with CTR, while STF resulted in a progressive significant decline in G' ; however, the FTC (1–5) samples were similar ($P > 0.05$) to CTR. At temperatures $>$ crossover temperature, TF had no impact on G' , but G'' significantly reduced in STF-moderate and -intense samples. The power-law factor for G' and G'' values from frequency sweep (10–100 rad/s; $\gamma = 0.5\%$, 55°C) tests indicated significant differences in the effects of rapid and slow TF. The STF and FTC significantly impacted intracycle large (G_L') and minimum strain (G_M') modulus and intracycle complex viscosity at large (η_L') and minimum (η_M') strain-rate obtained from large deformation rheology. As a result, strain-dependent zones of minor thickening ($T > 0$; $\gamma < 20\%$) to brief shear-thinning ($T < 0$; $\gamma > 30\%$) and 2 broad ranges for strain-stiffening (S) at $\sim 1 < \gamma < 100\%$ ($0 < S < 1.2$) and at $\gamma > 100\%$ ($\sim 0.8 < S < 1.8$) were identified for different TF schemes. Micro-scale cracks observed in the cheese matrix through confocal microscopy complimented the rheological observations. From high-field NMR experiments, the relaxation times and diffusion coefficient (D ; m^2/s) linked to protons (^1H) of water/fats in cheese indicated quantitative differences between the 2 TF schemes. This study addresses a critical knowledge gap of industrial relevance and adds to the available understanding about frozen storage of pasta-filata cheeses.

Key Words: cheese freezing, large deformation rheology, nuclear magnetic resonance (NMR)

1126 Enhanced upcycling of yogurt acid whey in a sports recovery beverage through activated carbon deodorization. V. Shukla* and O. I. Padilla-Zakour, *Cornell AgriTech, Geneva, NY.*

Yogurt acid whey (YAW) is a byproduct of the Greek yogurt industry which is typically discarded, due to low pH and yogurt taste, leading to massive waste and environmental concerns. However, it contains high amounts of electrolytes that may be useful for value-added beverages. Activated carbon has been used to remove undesirable volatile compounds from liquids. Surface functional groups may bind to compounds in the YAW that cause off-flavors, increasing acceptability. The electrolytes, acidity, and slight yogurt flavor makes YAW a suitable ingredient for sports recovery beverages (SRB) used by athletes for recovery after strenuous exercise. The YAW from 2 commercial yogurt facilities was used to create a ginger-lime flavored SRB (64% whey) at bench for chemical testing, then scaled to pilot scale, were created using native YAW and YAW that was batch treated with food-grade activated carbon (500 ppm) for 0, 5, 10, 20, 40 min at room temperature. The resulting samples (20 in total) were tested for pH, color, dissolved solids, salt content, conductivity, and proximate analysis. Additionally, the activated carbon was tested for point of zero pH and proximate surface functional groups. All studies were conducted in analytical duplicates of biological triplicates. A consumer hedonic sensory evaluation, an athlete hedonic rehydration evaluation, and a discrete choice analysis were also conducted. Characterization of YAW indicated that total

solids (6.7 to 6.6), salt (0.38 to 0.39%), and pH (4.29 to 4.28) were not significantly ($P > 0.05$) affected by the carbon treatment up to 40 min. Samples indicated a lightening and whitening in color with increase in carbon contact time ($P < 0.05$). Hedonic test (100 participants) results indicated a significantly ($P < 0.05$) higher preference for 5-min carbon contact time, with consumers indicating a deodorized, but not fully quenched, whey beverage being desirable. This aligned with overall liking (4.47 to 5.26) and aroma liking (5.00 to 5.44). Choice analysis indicated that clean label and pricing were significant ($P < 0.05$) to purchase intent. In the athlete study (31 participants), 65% of respondents perceived the beverage to be hydrating. This work shows that carbon treatment increases the acceptability of YAW based products without losses to desirable traits. It also indicates a use for YAW, a major wasted byproduct, in SRB that capitalizes on its inherent characteristics. The resulting characteristics of the SRB and ease of pilot-scale preparation indicate a strong suitability for adoption by industry as an upcycled value-added product.

Key Words: acid whey, sensory, upcycling

1127 Impact of whey protein phospholipid concentrate and its fraction supplementation on bone and muscle health in a weanling mouse model. M. T. Armstrong*¹, K. A. Gomes Vilela Antunes², N. Willis², J. F. Pierre², and G. Ozturk¹, ¹*Food Science, University of Wisconsin–Madison, Madison, WI*, ²*Nutritional Sciences, University of Wisconsin–Madison, Madison, WI.*

Nutrition plays a crucial role in developmental physiology, and dairy-derived macronutrients have shown positive effects on various health metrics, particularly bone health. A key component of dairy macronutrients is the milk fat globule membrane (MFGM), which serves as a carrier for biologically active molecules in the gastrointestinal tract. Whey protein phospholipid concentrate (WPPC) is an underutilized dairy stream produced during the production of whey protein isolate and is rich in bioactive macronutrients, including MFGM lipids and proteins. Although these components have been associated with beneficial physiological health outcomes, the direct impacts on bone and muscle health remain to be fully elucidated. We hypothesize that WPPC and its fractions are a valuable source of bioavailable components that maintain lean body mass, augment bone integrity, and enhance femur length. To explore these effects, we isolated lipids from WPPC by heating to 40°C to melt fat, followed by centrifugation at 4°C to separate the lipids. For the proteins, we performed 0.1 μm microfiltration followed by 30 kDa ultrafiltration to isolate MFGM proteins. We then fed 36 ($n = 9$) weanling mice an isocaloric diet supplemented with 10% lipid, protein, and whole WPPC compared with an isocaloric control (chow) diet. A 2-way ANOVA with post hoc Tukey's test ($P < 0.05$) was used to determine treatment effects over 4 time points: wk 0, 4, 8, and 12. After a 12-wk dietary intervention, we observed that body weight, fat, and lean mass were unchanged across the groups. However, bone imaging scans demonstrated significant increases in femur length—4.44% ($P = 0.0012$) in the protein-supplemented group and 4.01% ($P = 0.0068$) in the WPPC group by wk 12 of treatment. Additionally, the fat-supplemented group demonstrated a significant increase in bone mineral density of 6.55% ($P = 0.0008$). These findings suggest that supplementation with WPPC and its lipid and protein components differentially benefit developing femur length and bone mineral density in weanling mice as they transition into early adulthood. Our findings highlight the potential applications of WPPC in enhancing childhood nutrition strategies.

Key Words: whey, bone development

Animal Health Symposium: Interspecies Crossover of Highly Pathogenic Avian Influenza into Dairy Cattle

1128 Combating the complex ecology of highly pathogenic avian influenza virus: Inoculation to application in lactating dairy cattle. B. Arruda^{*1}, A. L. Baker¹, M. B. Palmer¹, P. Boggiatto¹, C. Souza¹, B. Trindade¹, G. C. Zanella¹, A. Campos¹, K. Lantz², M. Torchetti², P. J. Gorden³, and K. Sarlo Davila¹, ¹*Agriculture Research Service, United States Department of Agriculture, Ames, IA*, ²*United States Department of Agriculture, Animal and Plant Health Inspection Service, Ames, IA*, ³*College of Veterinary Medicine, Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA*.

Influenza A viruses (IAV) remain the most common cause of pandemics. Highly pathogenic avian influenza A (HPAI) strains belonging to the H5NX subtype have pandemic potential and the ecology of currently circulating H5N1 viruses has become increasingly complex due to factors previously not documented. This presentation will provide information on the complex ecology of IAV including HPAIV, viral traits that promote infection and transmission, the factors that influence virus-host interactions and expression of clinical disease, and pathology. The molecular, serologic, and pathology results from 6 inoculated lactating dairy cows and 2 convalescent lactating dairy cows and the application of this data to the field and vaccine evaluation will be discussed.

1129 The impact of influenza A H5N1 virus infection on dairy production and its risks to public health. D. G. Diel^{*1,2}, F. Pena-Mosca³, M. Nooruzzaman¹, E. Frye^{1,2}, N. H. Martin⁴, M. MacLachlan¹, S. D. Alcaine², P. S. B. de Oliveira¹, M. Zurakowski², M. K. Koeve¹, R. Ivaneck¹, M. Prarat-Koscielny⁵, Z. R. Lieberman⁶, W. L. Leone^{1,2}, and F. Elvinger^{1,2}, ¹*Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY*, ²*Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University, Ithaca, NY*, ³*Department of Public and Ecosystem Health, College of Veterinary Medicine, Cornell University, Ithaca, NY*, ⁴*Department of Food Science, College of Agriculture and Life Sciences, Ithaca, NY*, ⁵*Ohio Animal Disease and Diagnostic Laboratory, Reynoldsburg, OH*, ⁶*College of Veterinary Medicine, Cornell University, Ithaca, NY*, ⁷*Forthright Dairy Consulting, Wayne, OH*.

The spillover of highly pathogenic avian influenza (HPAI) H5N1 virus into US dairy cattle poses significant challenges to the industry and potential public health risks. We investigated an HPAI H5N1 outbreak's impact on dairy cattle and evaluated virus stability in dairy products. In an affected herd of 3,876 adult cows, 20.0% exhibited clinical disease lasting 3 wk, while herd seroprevalence reached 89.4%, with 76.1% of infections being subclinical. Clinically affected cows demonstrated a 6-fold higher mortality risk and 3.6-fold increased risk of premature herd removal, with milk production losses averaging 900 kg per cow during the 60-d post-outbreak period. Economic losses, including decreased milk production, mortality, and early herd removal, averaged \$950 per affected cow, totaling approximately \$737,500 for the herd. Analysis of virus stability in raw milk showed effective inactivation (5–6 log reduction) under standard pasteurization and most thermization conditions, except at 50°C for 10 min. In raw milk cheese studies, virus survival was pH-dependent; infectious virus persisted through cheese making and aging for up to 60 d at pH 6.6 and 5.8 but was inactivated during cheese making at pH 5.0. These findings, confirmed in commercial raw milk cheese samples, demonstrate substantial production and economic

impacts while highlighting the effectiveness of thermal treatments and the potential use of thermization of milk pH control in cheese making for ensuring dairy product safety.

Key Words: influenza A, dairy cow, mastitis, economic impact, food safety

1130 H5N1 through the lens of time: Before, during, and after the outbreak in dairy cattle. G. A. Wager-Jones^{*1,2}, J. Hague¹, K. Lotspeich¹, C. Hamilton¹, and M. Fujiwara¹, ¹*Peacock Technology, Stirling University Innovation Park, Stirling, United Kingdom*, ²*Harper Adams University, Newport, Telford, United Kingdom*.

The study assessed variation in milk yield (MY) and body condition score (BCS) during an H5N1 outbreak. A total of 1,032 observations from 172 cattle were collected between October 15 and December 2 across 6 dates. The first confirmed infection was on October 19. The MY data came from farm software, while BCS was assessed via images. Data included lactation number and days in milk (DIM). The BCS was classified as under-conditioned (UC) (<2.5), normal-conditioned (NC) (2.5–3.5), or over-conditioned (OC) (>3.5). Two robust linear mixed models addressed data non-normality. Fixed effects included BCS category, date, and their interaction, with cows as a random effect. The model explained 74% of MY variation, with 29% attributed to fixed effects. Interaction effects showed MY changes over time by BCS. On October 22, UC and OC cows had no significant MY changes. By October 28, UC cows showed a significant MY drop (–15.5 lb, $t = -4.5$, $P < 0.001$), whereas OC cows had a non-significant reduction (–8.2 lb). By November 11, MY decline persisted in UC cows (–8.8 lb, $t = -2.5$, $P = 0.014$) but remained minor in OC cows (–3.8 lb). By December 2, MY losses stabilized (–3.5 lb for OC, –5.1 lb for UC). A separate model analyzed BCS, explaining 90.7% of its variation, with 7% due to fixed effects. Post-outbreak, BCS dropped 0.03 on October 28 ($t = -2.4$, $P < 0.05$) but recovered, increasing by 0.05 on November 11 ($t = 3.4$, $P < 0.001$), 0.06 on November 18 ($t = 3.8$, $P < 0.001$), and 0.11 on December 2 ($t = 7.0$, $P < 0.001$). H5N1 had no immediate effect, but MY and BCS declined from October 28. The BCS recovered, but MY continued decreasing until December 2. The UC cows suffered the most severe MY losses, likely due to metabolic stress, whereas OC cows experienced a milder, temporary decline. Both groups produced less milk post-infection. Continuous BCS monitoring may aid recovery, especially for UC and OC cows.

Key Words: H5N1 outbreak, automated body condition score (BCS), disease recovery monitoring

1135 Transmission of highly pathogenic avian influenza H5N1 to calves fed unpasteurized milk from experimentally infected cows. K. M. Sarlo Davila^{*}, A. L. Baker, P. M. Boggiatto, M. V. Palmer, E. J. Putz, S. C. Olsen, G. C. Zanella, A. Campos, A. Buckley, and B. Arruda, *USDA-ARS National Animal Disease Center, Ames, IA*.

Highly pathogenic avian influenza (HPAI) was confirmed in dairy cattle in Texas on March 25, 2024, by the US Department of Agriculture (USDA) National Veterinary Services Laboratories (NVSL) in response to a multi-state investigation into milk production losses. Infectious virus and viral RNA were consistently detected in milk from affected cows.

The amount and duration of virus shed in milk from inoculated mammary quarters point to milk as a primary source of virus spread within and between dairy herds. Pasteurization has been shown to inactivate the virus in milk; however, domestic cats consuming raw milk from affected cows developed fatal systemic influenza infection. To determine if HPAI could be transmitted to calves fed unpasteurized milk from virus positive lactating cows 4 Holstein calves of approximately 7 to 11 wk old were fed 0.95 L of unpasteurized milk from experimentally inoculated cows twice a day via bucket. Calves developed clinical signs including nasal discharge, mild fever, mild lethargy, loose stool, and slightly increased respiratory effort for 5–6 d. Viral RNA was consistently detected in nasal swabs from all 4 calves from 2 DPI through 4 DPI, with consistent detection persisting after calves were transitioned to being fed milk from non-inoculated cows. Infection was confirmed by viral RNA detected in nasal swabs, lung lesions, antigen and RNA detection in lung, multiple lymph nodes and pharyngeal tonsil, and seroconversion.

Key Words: transcriptomics, immunology, influenza

1136 Frequency and treatment of highly pathogenic avian influenza A (H5N1) in high, average, and low immune responder cows. S. C. Beard^{*1}, M. E. Carson¹, B. Mallard^{2,3}, M. Lohuis^{1,3}, and F. Malchiodi^{1,3}, ¹*Semex Alliance, Guelph, ON, Canada*, ²*Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada*, ³*Centre for the Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada*.

Avian influenza A (H5N1) was first identified in 1959, but it was not until 2020 that a new variant, termed highly pathogenic avian influenza (HPAI), emerged. Initially detected in wild and domestic birds in Europe, it spread to North America in 2021. In 2024, HPAI A (H5N1) outbreaks spread to dairy cattle, presenting significant health challenges and industry instability. Semex's Immunity+ is a genetic breeding solution designed to enhance broad-based disease resistance in dairy cattle by identifying bulls and cows with superior immune response. This selective breeding approach has led to significant reductions in disease incidence, including but not limited to mastitis, pneumonia, and lameness, contributing to improved herd health and increased profitability for producers. The objective of this study was to evaluate the relationship between Immunity+ genomic estimated breeding value (GEBV) and the probability of requiring treatment for HPAI A (H5N1) over a 12-mo period. Treatment records and Immunity+ GEBVs were collected for 15,386 cows from 6 large commercial dairy farms in the United States that experienced HPAI outbreaks. Cows were classified as "High," "Average," and "Low" responders based on their Immunity+ GEBV. A binomial logistic regression and ANOVA was conducted to assess the association between immune response GEBV and HPAI treatment probability. Odds ratios and marginal means were estimated to quantify treatment likelihood. Differences in treatment probability among response groups were evaluated using Tukey's adjustment for multiple comparisons. Results indicated that cows classified as "Low" responders had a significantly higher probability of requiring treatment for HPAI compared with those classified as "High" responders ($P = 0.02$, odds ratio = 1.16). It is probable that high responders were equally exposed to HPAI A (H5N1), but did not develop signs severe enough to require treatment, suggesting greater resistance to disease. These results indicate that genetic selection for immune responsiveness can significantly reduce HPAI A (H5N1) treatment rates in commercial dairy cattle herds, providing a sustainable strategy for enhancing herd

health, reducing disease burden, and mitigating the economic impact of outbreaks of HPAI in dairy production systems.

Key Words: immune response, dairy, genetics

1137 Factors associated with risk of clinical signs of highly pathogenic avian influenza infection and impacts on productivity in lactating dairy cattle. L. Olthof^{*1}, K. C. Krogstad², and B. J. Bradford¹, ¹*Department of Animal Science, Michigan State University, East Lansing, MI*, ²*Department of Animal Science, The Ohio State University, Wooster, OH*.

As highly pathogenic avian influenza (HPAI) spreads in US dairy herds, substantial uncertainty remains regarding mode(s) of transmission and net impacts on cattle health and productivity. Therefore, the objectives of our study were to identify factors associated with risk of HPAI diagnosis on-farm among lactating cows and quantify subsequent productivity of HPAI+ vs. HPAI- cows. We utilized herd management data from a farm in Michigan (47.4 ± 0.2 kg/d ECM, 1.58 ± 0.04 SCS) that experienced an HPAI outbreak in May 2024. Over 13 d, 20.4% of the herd's lactating cows (693/3,391) exhibited clinical symptoms and were treated for HPAI. Univariate analysis (GLIMMIX) demonstrated greater risk of diagnosis for multiparous vs. primiparous cows (odds ratio [OR] 1.81; 95% CI 1.49–2.20); pregnant vs. not (OR 1.34; 1.13–1.59); post-peak vs. <100 DIM (OR 1.51; 1.26–1.80); and greater milk yield (OR 1.012 per kg/d; 1.003–1.022). Multivariate modeling retained only parity and the quadratic effect of DIM (both $P < 0.01$) as risk factors, with peak risk for lactation 5 and 186 DIM. Impacts of HPAI+ diagnosis in the same lactation were assessed with data from 9 test days through January 2025 in a model that included parity, the quartic fit of DIM, and repeated measures within cow. At 25 d after onset, HPAI+ cows produced 14.8 ± 0.44 kg/d ($P < 0.001$) less ECM and SCS increased 1.91 ± 0.08 units ($P < 0.001$) relative to HPAI- cows. Although SCS no longer differed at 98 d post-onset, ECM yield remained significantly different through 224 d after the outbreak (-4.24 ± 0.71 kg/d, $P < 0.001$). Primiparous cows had less ECM reductions than multiparous cows (-2.21 ± 0.65 vs. -7.16 ± 0.73 kg/d for parity 1 vs. 3, $P < 0.001$). Fat and protein concentrations increased subtly for 2 and 4 mo post-onset, respectively. In summary, primiparous appear to have been somewhat protected from clinical HPAI and suffered fewer production losses than multiparous cows. Milk production was impaired for more than 7 mo after HPAI onset, far longer than impacts on SCS.

Key Words: highly pathogenic avian influenza (HPAI), H5N1, disease

2210 Enhancing dairy industry preparedness: A collaborative outreach approach to managing highly pathogenic avian influenza (HPAI) in California. N. Silva-Del-Rio^{*1}, R. Branco Lopes², D. R. Bruno³, B. M. Karle⁴, R. A. Black⁵, M. Lema⁶, and M. Payne^{7,8}, ¹*School of Veterinary Medicine, University of California–Davis, Tulare, CA*, ²*University of California Agriculture and Natural Resources, Cooperative Extension Tulare County, Tulare, CA*, ³*University of California Agriculture and Natural Resources, Cooperative Extension Fresno County, Fresno, CA*, ⁴*University of California Agriculture and Natural Resources, Cooperative Extension Glenn County, Orland, CA*, ⁵*University of California Agriculture and Natural Resources, Cooperative Extension Sonoma County, Santa Rosa, CA*, ⁶*California Milk Advisory Board, Tracy, CA*, ⁷*Western Institute of Food Safety and Security, University of California–Davis, Davis, CA*, ⁸*California Dairy Quality Assurance Program, Davis, CA*.

The highly pathogenic avian influenza (HPAI) outbreak has posed a significant challenge for California's dairy industry. To support dairy producers and the allied industry, several outreach efforts were organized by UC Cooperative Extension (UCCE). A webinar series, held from September–December 2024 in partnership with the California Milk Advisory Board and the California Dairy Quality Assurance Program, featured updates from the California Department of Food and Agriculture (CDFA), HPAI overview (e.g., clinical signs, transmission routes, preliminary epidemiological findings), treatment strategies, dairy producers' and veterinarians' perspectives on HPAI's management, financial assistance programs, and research updates. Each session averaged 1 h and included a Q&A session. In a parallel effort, UCCE launched a 3-location workshop series in Northern California, focusing on preventive practices, as the outbreak had not yet reached this region. The in-person workshops attracted 80 attendees, including producers and allied industry. Our assessment of knowledge gained was informal, based on direct conversations with attendees after the event. All participants we spoke with indicated an increase in understanding of the topic. We selectively targeted the dairy producers that attended those meetings for follow-up conversations; but we were less interactive with allied industry participating in the meetings. The team also developed written outreach materials (one magazine article, one newsletter article, and 3 fact sheets) to provide timely information to dairy producers. A formal needs assessment evaluating the impact of these educational efforts is underway. Additionally, the team is part of the Extension Collaborative on Immunization Teaching and Engagement (EXCITE) initiative. Within EXCITE, the H5N1 program aims to increase dairy producers' knowledge of HPAI's impact on human health and promote immunization of dairy workers. The team has collaborated with local and state Human Health Services (HHS) to gather information on rural vaccination clinics and their efforts in disease prevention and dairy producer education. As part of this initiative, a pilot program was launched in January 2025 in one county, with UCCE, the Farm Bureau, and HHS collaborating on a vaccination clinic. In summary, UCCE approached HPAI outreach efforts as a One Health issue, ensuring the protection of both animal and human health through collaborative and multi-disciplinary efforts within California's dairy industry.

Key Words: highly pathogenic avian influenza (HPAI), dairy industry outreach

1139 Impact of HPAI and its role in strengthening biosecurity practices in dairy farming. Z. Rodriguez*, *Large Animal Clinical Science Department, College of Veterinary Medicine, Michigan State University, East Lansing, MI.*

The recent outbreak of highly pathogenic avian influenza (HPAI) H5N1 in cattle has highlighted the vulnerabilities of the dairy sector to the entrance of emerging infectious diseases and reinforced the need for proactive preparedness against novel and endemic threats. While many aspects of the disease remain under study, the experience acquired from over a year of dealing with HPAI H5N1 in cattle has provided valuable insights into its prevention and impact. To better inform HPAI H5N1's preparedness, and develop cost-effective prevention and control strategies, is imperative to quantify its impact on animal productivity. These insights help farmers and advisors make informed decisions on disease management, treatments, and resource allocation while guiding policy-makers in designing policies, subsidies, and compensation mechanisms to mitigate economic losses. Furthermore, recognizing HPAI H5N1's impact may also empower farmers to adopt stronger preventive measures. Typically, biosecurity measures applied in dairy farms have been highly inconsistent across operations as they depend on multiple factors, such as farmer's risk preconceptions, posing a challenge to disease management efforts. While farm-to-farm transmission requires further study, strengthening biosecurity and updating them based on research findings is the most sensitive approach to reduce disease spread and enhance industry resilience. This presentation will focus on 2 key aspects: HPAI H5N1's productive and economic impact and farmers' willingness to adopt stricter biosecurity measures.

Key Words: highly pathogenic avian influenza (HPAI), H5N1, dairy cattle, economic impact

Joint Animal Behavior and Well-Being and Forages and Pastures Symposium: Societal and Production Implications of Pasture Access for Dairy Heifers

1140 The alignment of societal expectations for providing outdoor access to dairy cows and their welfare. P. D. Krawczel^{*1,2}, A. M. K. Sjöholm^{1,3}, T. Kokkonen^{1,3}, I. Herzon^{1,4}, P. J. Rissanen¹, and A. O. Vanhatalo^{1,3}, ¹*Department of Agricultural Sciences, Faculty of Agriculture and Forestry, University of Helsinki, Helsinki, Uusimaa, Finland*, ²*Department of Production Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Uusimaa, Finland*, ³*Helsinki One Health, University of Helsinki, Helsinki, Uusimaa, Finland*, ⁴*Helsinki Institute for Sustainability Sciences, University of Helsinki, Helsinki, Uusimaa, Finland*.

One aspect of an animal production system maintaining its social license to operate is ensuring production practices align with societal values. The expansion and intensification of dairy production across much of the western world has contributed to the reduction of grazing, or outdoor access in general, for lactating dairy cows. This is contrary to the public image of idyllic dairy cows grazing on green pastures, which is also often used in media or advertising of dairy products. This misalignment of public perception and the current production practices may result in a loss of public trust. However, simply providing increased outdoor access does not necessarily improve animal welfare either as going outdoors under some circumstances is detrimental to welfare. Reduced biological function, generally from increasingly variable diet, is often one of the main welfare issues and reasons for farmers' hesitancy to incorporate outdoor access as a routine management strategy. Additionally, increased labor needs may limit outdoor access for cows. Therefore, outdoor access presents an interesting challenge within modern dairy production as it is generally expected by the public, but its role in meaningfully improving dairy cow welfare is not necessarily straightforward. The objective of this review is to address public attitudes toward outdoor access for dairy cows, the challenges and opportunities stemming from providing outdoor access to improve welfare, and how to align these factors to maintain public trust in dairy production. To address public perception, this review will compare and contrast the views of the general public and those of farmers to understand where agreement can be found. Furthermore, possible cultural or farm-based explanations will be explored for regional differences in outdoor access/pasture use. Regarding welfare, this review will explore the factors, including quality of transfer lanes, feed preferences, and heat stress, that can positively or negatively affect the welfare of cows while outdoors. Finally, we will investigate how this collectively fits together to promote understanding the alignment between the factors of outdoor access valued by the public, those valued by farmers, and those that contribute to improved dairy cow welfare.

Key Words: welfare, society, outdoor

1141 Pasture versus confinement for dairy heifers: Testing assumptions about well-being based on interpretations of behavior. M. B. Hall^{*1}, D. Jaramillo², K. F. Kalscheur¹, C. H. Paiva Camisa Nova³, and A. DiSalvo⁴, ¹*US Dairy Forage Research Center, USDA-ARS, Madison, WI*, ²*US Dairy Forage Research Center, USDA-ARS, Marshfield, WI*, ³*Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI*, ⁴*Westlake High School, Thornwood, NY*.

Assessment of animal well-being requires identifying attributes related to an animal's status. Reduced well-being has been associated with

increased stereotypy, vocalization, and cortisol levels. Do these assessments of stress agree with each other? The impact of rearing environment on behavior and hair cortisol levels in growing Holstein heifers (Hf) starting at 4.8 mo of age (standard deviation [SD]: 0.05; body weight 166, SD: 1 kg) and continuing over 2 years was studied. The Hf were raised from May to August on rotationally stocked cool-season pastures supplemented with grain and/or a vitamin and mineral mix (PAS) or raised in a barn and fed a total mixed ration (BARN). The Hf were assigned to one of 8 treatment (Trt) groups: 4 PAS and 4 BARN groups. For reasons unrelated to the study, 1 Hf was removed from each Trt. Before the study and outside of pasture season, all Hf were housed in a barn. Yearly, hair samples were obtained in May before pasturing, June, and August; behaviors were recorded by trained observers every 10 min for 12 h during daylight on 1 d each in June and July. Behavior data were converted to episodes per Hf by dividing observations by number of Hf in a group. Data were analyzed as repeated measures in a model with Trt, sampling date, year, and interactions, and with group as a random variable and the experimental unit. Standard errors in italics are not back transformed (Table 1). For cortisol, May data were used as a covariate but not significant ($P = 0.20$). Stereotypy was greater for BARN than PAS in both years. Vocalization was greater for PAS in Year 1 and associated with feeding time, and for BARN in Year 2. Hair cortisol was greater for PAS in both years. Hair cortisol measures did not agree or consistently agree with stereotypy and vocalization measures. Corroboration of behavioral measures that align with indicators of animal stress is needed.

Key Words: well-being, behavior, heifer

1142 Mitigating heat stress on pasture. C. B. Tucker^{*1} and K. E. Schütz², ¹*Center for Animal Welfare, Department of Animal Science, Davis, CA*, ²*AgResearch Ltd., Hamilton, New Zealand*.

Heat stress in dairy cattle influences efficiency, animal welfare, and sustainability. Strategies to reduce heat load will depend on breed, location, housing and production levels. On pasture, a consistent and cost-efficient way to mitigate heat stress is to provide natural or man-made shade. We will review the scientific evidence about how motivated cattle are to access shade. We will discuss how the quality and availability of shade influences use and effectiveness. Proximity of shade to other resources, such as drinking water, are also important considerations. Indeed, access to water also plays a role in mitigating heat stress. Water quality and availability are important considerations in all housing and we will

Table 1 (Abstr. 1141). Results of study

Measure	Stereotypy episodes/heifer	Vocalization episodes/heifer	Hair cortisol, pg/mg
Barn year 1	2.1	0.19	4.8
Pasture year 1	0.09	0.99	15.4
Barn year 2	3.3	0.40	4.4
Pasture year 2	0.01	0.27	18.9
Standard error	0.12	1.05	0.0199
P : Trt, Trt $\times$ Year ¹	<0.01, 0.01	0.26, 0.01	<0.01, <0.01

¹Trt = treatment.

discuss the specific implications on pasture (e.g., grouping around the trough preventing access; challenges of proving functional water sources in daily rotational grazing systems). In addition to shade and water provision, other possible management tools for nonlactating cattle include shifting the time of feeding in rotational grazing systems to reduce heat load. Last, we will discuss when heat mitigation is needed and methods to assess if the strategies employed are effective. We will cover both environmental-related metrics including those that aggregate relevant weather conditions (e.g., temperature, wind, solar radiation, humidity) and animal-based outcomes (e.g., respiration rate, drool, panting). Our review will highlight the existing scientific knowledge about mitigating heat stress in cattle on pasture and where further research is needed.

Key Words: heat stress, cattle, shade

1143 Impact of pasture versus confinement rearing on dairy heifer growth and transition period. C. H. P. Camisa Nova^{*1}, D. M. Jaramillo², M. S. Akins², and K. F. Kalscheur³, ¹*University of Wisconsin–Madison, Madison, WI*, ²*US Dairy Forage Research Center, Marshfield, WI*, ³*US Dairy Forage Research Center, Madison, WI*.

The goal of raising dairy heifers is optimal growth to calve by 22–24 mo of age while minimizing costs. This study evaluated the effects of raising dairy heifers on pasture (PAST) compared with confinement (CONF) on growth as a heifer, and intake during the transition period. Sixty-two Holstein heifers (5.2 ± 0.45 mo old) were raised under 2 practices: (1) 31 heifers (3 pastures of 8 and 1 pasture of 7 heifers) were rotationally stocked on legume-grass mixed pastures for 5 mo during 2 grazing seasons, and (2) 31 heifers (3 pens of 8 and 1 pen of 7 heifers) were housed and fed grass- and corn silage-based TMR diets. Diets were formulated for heifer growth to reach 94% of mature weight at first calving. Body weight (BW), hip height (HH), withers height (WH), heart girth (HG), body length (BL), and hip width (HW) were taken through the 2 years of the trial. A logistic growth curve $\{Y = A / (1 + \exp[-B(t-C)])\}$ was set for each variable and parameters of the model statistically compared between the treatments. During the transition period, BW and body condition score (BCS) were assessed from –21 through 21 d relative to calving (23 ± 0.8 mo old). Dry matter intake (DMI; % BW) was recorded before calving (wk –4 to –1 relative to calving), and data were calculated as percentage of BW for 21 d after calving. There were no differences between treatments for parameters in the logistic growth model for BW, WH, HG, BL, and HW ($P > 0.05$). For HH, the parameters A (PAST: 144.4 vs. CONF: 141.1 cm, $P = 0.032$) and B (PAST: 0.1306 vs. CONF: 0.1646 cm, $P = 0.044$) differed between treatments. This difference may be attributed to the fact that the animal groups were balanced only based on BW, but the groups were similar for HH before calving. During the transition period, BW was similar between the groups (PAST: 625 vs. CONF: 640 kg, $P = 0.15$) whereas BCS was affected by treatment during the transition period (PAST: 3.34 vs. CONF: 3.47, $P = 0.006$). For DMI, PAST had greater intake than CONF (2.11 vs. 1.95% BW/d, $P = 0.002$). Thus, grazing dairy heifers can achieve similar growth at transition as confined heifers, while exhibiting increased DMI before calving.

Key Words: heifer development, performance, grazing

1144 Plasma folate and vitamin B₁₂ concentrations of heifers raised indoors or on pasture. M. Duplessis^{*1}, M. B. Hall², D. M. Jaramillo², K. F. Kalscheur³, and C. H. Paiva Camisa Nova⁴, ¹*Agri-culture and Agri-Food Canada, Sherbrooke, QC, Canada*, ²*US Dairy Forage Research Center, USDA-ARS, Madison, WI*, ³*US Dairy Forage*

Research Center, USDA-ARS, Marshfield, WI, ⁴*Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI*.

Folates (B₉) and vitamin B₁₂ (B₁₂) are involved in protein and energy metabolism. Our objective was to evaluate plasma B₉ and B₁₂ concentrations of Holstein heifers raised on pasture and rotationally stocked on cool-season pastures and supplemented with grain with a vitamin and mineral mix (PAS) or raised in a barn and fed a total mixed ration (BARN). Before the start of the study, all heifers were housed in a barn. Heifers ($n = 64$) weighing 166 [standard deviation (SD): 1] kg were assigned to one of 8 groups at 4.8 (SD: 0.5) months: 4 PAS and 4 BARN groups. A PAS heifer died due to cause unrelated to the study. Plasma samples were collected by caudal venipuncture in May before treatments began (covariate) and in July, 2 mo after treatments began. Plasma B₉ and B₁₂ were analyzed by chemiluminescence. The PROC MIXED of SAS was used to separately analyze plasma B₉ in May and July with treatment as fixed effect. For July, plasma B₉ in May was a covariate. As some samples were below the detection limit of 150 pg/mL for B₁₂, the proportion of samples below the detection limit was analyzed using PROC GLIMMIX of SAS (chi-squared test) with treatments as fixed effect, separately for May and July. Group was the experimental unit. Values are expressed as least squares means $\pm$ standard error. In May, plasma B₉ averaged 7.19 ± 0.25 ng/mL and did not differ between treatments ($P = 0.39$). In July, plasma B₉ tended to be higher with PAS (7.29 ± 0.24 ng/mL) than BARN (6.59 ± 0.24 ng/mL; $P = 0.10$). The proportion of heifers with plasma B₁₂ below the detection limit averaged $75 \pm 8\%$ in May with no difference between treatments ($P = 0.63$). In July, the proportion of animals below the B₁₂ detection limit was greater for PAS ($63 \pm 9\%$) than for BARN ($35 \pm 9\%$; $P = 0.03$), suggesting that overall plasma B₁₂ for PAS heifers was lower than for BARN. In conclusion, although dry matter intake was not evaluated, our results suggested that the diets plus environment affected plasma B₉ and B₁₂ statuses of Holstein heifers. More research should be conducted to confirm these results and evaluate if they have a practical implication.

Key Words: cattle, grazing, cyanocobalamin

1145 Milk fatty acids of Holstein cows compared with Holstein, Jersey, Montbéliarde, Normande, and Viking Red crossbred cows in an organic grazing and low-input conventional research dairy herd. K. M. Bosley^{*}, L. B. Hansen, and B. J. Heins, *University of Minnesota, St. Paul, MN*.

There is growing consumer interest in milk with perceived health benefit from fatty acids (FA), which may open marketing opportunities for producers. The objective of the study was to compare milk FA between Holstein (HO; $n = 166$) and crossbred dairy cattle composed of Montbéliarde (MO), Viking Red (VR), and HO breeds (MVH; $n = 516$) and Normande (NO), Jersey (JE), and Viking Red (VR) breeds (NVJ; $n = 233$) at the University of Minnesota West Central Research and Outreach Center (WCROC; Morris, MN) low-input and organic dairy herds. Total, de novo, mixed, preformed, and individual FA were from monthly milk samples from Minnesota DHIA (Buffalo, MN) and data were from February 2021 to June 2024. Test date observations were used to fit Legendre polynomial random regression lactation curves to compare 305-d FA. Primiparous and multiparous lactation curves were evaluated separately. Each cow and lactation was required to have 3 test dates. For statistical analysis, fixed effects were breed group, sire breed within breed group, lactation number (first, second, or third and greater), herd (low-input or organic), calving season (spring or fall) nested within herd, test-date month, and the interactions of lactation and breed group, herd and lactation, test-date month and herd, and lactation

and test-day month. Random effects were cow nested within breed group and herd and days in milk at test day. The organic herd had higher ($P < 0.05$) preformed (37.8 vs. 35.3 g/100 g total FA), 18:0 (11.4 vs. 10.6 g/100 g total FA), and 18:1 *cis*-9 (20.6 vs. 19.6 g/100 g total FA) FA than the low-input herd. Both crossbred (MVH and NJV) groups had higher ($P < 0.05$) de novo (25.9 and 26.0 vs. 25.4 g/100 g total FA) FA and lower 16:0 (35.2 and 34.7 vs. 35.6 g/100 g total FA) FA than HO.

Phenotypic correlations between 305-d FA and average SCS, days open, and survival did not indicate negative impacts of selection for improved FA production. Crossbreeding along with pasture feeding of cows may improve milk FA for potential niche markets.

Key Words: milk fatty acid, crossbreeding, organic

Production, Management, and the Environment 1

1146 Feeding strategies for greenhouse gas mitigation using the DairyPrint Model. C. L. Huang* and V. E. Cabrera, *University of Wisconsin–Madison, Madison, WI.*

Greenhouse gas (GHG) emissions from dairy farming, particularly enteric methane, significantly affect livestock agriculture's environmental footprint. Establishing baseline emissions is essential for evaluating mitigation strategies. This study employed the DairyPrint model to estimate baseline GHG emissions for a herd of 570 lactating cows at the Arlington Agricultural Research Station, University of Wisconsin–Madison, which supplements monensin 450 mg per cow per day. Data included herd characteristics, dietary composition, and manure management, with diet formulations refined using NASEM (2021). Key dietary parameters included crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), crude fat (CF), and phosphorus (P). It was presumed that the use of monensin would decrease 5.5% methane emissions. Simulations estimated methane production at 418 g/day per cow, with a methane yield of 16.4 g/kg dry matter intake (DMI) and intensity of 10.3 g/kg milk. Precision feeding via nutritional grouping (NG) was evaluated as a mitigation strategy. Further research will refine grouping strategies and assess economic implications to support data-driven policies for sustainable dairy farming. This will involve clustering cows into 3 groups based on net energy for lactation (NEL), days in milk (DIM), and milk yield. Diets will be adjusted to better match each group's energy needs. The DairyPrint model will then be used to compare the level of methane emission reductions to the baseline. These results will highlight NG as a promising approach to reducing the environmental impact of dairy farming while simultaneously maintaining productivity and profitability.

Key Words: DairyPrint model, greenhouse gas mitigation, dairy sustainability

1147 Economic feasibility of voluntary carbon markets for methane-reducing feed additives in the US. F. Ferreira* and D. McCabe, *Clean Air Task Force, Boston, MA.*

Methane-reducing feed additives (MFA) are an important tool to reduce emissions from livestock, but their impact is dependent on economics. Voluntary carbon markets (VCM) may present an economic opportunity for farmers using MFA. Our goal was to evaluate the economics of selling carbon credits (CC) in the VCM for dairy farmers using MFA in the US, using 3-NOP as baseline, the only MFA approved for use in lactating dairy cows in the US. For a 1,000-lactating-cow dairy, a partial budget analysis was done to calculate the income over feed cost (IOFC) of using MFA, accounting for CC revenue from VCM (average CC of \$10/ton of CO₂ equivalent (CO₂e), global warming potential of methane = 28). No change in production, dry matter intake (DMI), or milk components were assumed. We modeled revenue from milk (average 34 kg/lactating cow per day using Holstein US averages and 0.44/kg of milk sold), feed costs (diet neutral detergent fiber [NDF] = 32.9%, average DMI = 23.9 kg, feed costs = \$5.40/lactating cow per day using Pennsylvania's average feed costs for 2023), 3-NOP effectiveness and costs (average 30% methane emissions reduction, cost = 0.39/lactating cow per day based on EU price plus a 30% markup for short-term importing costs). Daily methane emissions were estimated using the formula $CH_4 = -126 + 11.3 \times DMI + 2.3 \times NDF + 28.8 \times MF + 0.148 \times BW$ (3.8% milk fat, 626 kg mean BW). Total IOFC when no MFA was used \$9,660/day. When MFA was used, an extra cost of \$390/day was incurred, and 3.5

tons of CO₂e were not emitted per day, generating an income of \$35/day from CC sold in the VCM; therefore, IOFC for a farm using MFA was \$9,305/day. Sensitivity analysis demonstrated that CC price would have to be \$110/ton of CO₂e, or the cost of MFA would have to be \$0.04/cow per day for farmers to break even. Under current VCM conditions, farmers would not break even, even if MFA efficiency was increased to 100%. Further analysis should evaluate the effects of potential changes in DMI and milk components. This analysis demonstrated the need to incorporate farm-level economic analysis when environmental solutions are designed and implemented in the United States.

Key Words: methane-reducing feed additive, voluntary carbon market, economics

1148 Global warming from greenhouse gas emissions of Spanish dairy cattle: A historical perspective (1865–2021). A. del Prado^{*1,2}, G. Pardo¹, L. Lassaletta³, A. Sanz-Cobena³, and E. Aguilera⁴, ¹*Basque Centre for Climate Change (BC3), Leioa, Spain*, ²*Ikerbasque—Basque Foundation of Science, Bilbao, Spain*, ³*CEIGRAM-Universidad Politécnica de Madrid, Madrid, Spain*, ⁴*IEGD-CSIC, Madrid, Spain*.

There is an almost linear relationship between the cumulative amount of carbon dioxide (CO₂) emitted and the resulting global temperature. For non-CO₂ GHGs, we can approximate a similar linear relationship by converting long-lived GHGs (e.g., nitrous oxide: N₂O) and short-lived GHGs (e.g., methane: CH₄) into CO₂-equivalent emissions (CO₂-e) using the “Global Warming Potential” (GWP) metric at a 100-year horizon (GWP₁₀₀) and the GWP* metric (GWP star), respectively. In this study, we analyzed the temperature increase (using CO₂-e as a proxy) caused by direct dairy cattle emissions of non-CO₂ GHGs through enteric fermentation and manure management in Spain (1865–2021 period). The Spanish dairy cattle sector is estimated to have emitted a total of 296 Mt CO₂-e (17.7 Mt CH₄; 0.12 Mt N₂O) since 1865. Methane emissions account for approximately 89% of the total CO₂-e emissions. Historically, the share of warming attributable to manure CH₄ has increased significantly in recent decades, rising from below 5% of total CH₄ emissions to nearly 30%. The period with the most substantial increase in warming corresponds to the decades from 1970 to 1990, accounting for approximately 57% of the historical added warming. This increase also coincided with the fastest rise in milk production. Since 1990, although milk production has continued to rise slightly, the use of higher-yielding cattle and improvements in feed management have led to a decrease in both enteric and total CH₄ emissions intensity. Consequently, the warming from direct GHG emissions has begun to reverse. If the additional global warming from CH₄ emissions had been assessed using the GWP₁₀₀ metric, the warming from historical direct emissions would have been overestimated by 47%. Based on this, adopting GWP* over a sufficiently long-time series of CH₄ emissions provides a more accurate quantification of the historical warming caused. However, to fully understand the historical warming attributable to the Spanish dairy cattle, it is essential to quantify GHG emissions beyond direct sources (i.e., indirect emissions, mainly pre-farm emissions related to feed production).

Key Words: GWP*, Spain, climate impact

1149 Strategies for mitigating greenhouse gas emissions from US dairy farms toward a net zero goal. A. Rotz¹, K. Briggs^{*2}, A. N. Hristov³, A. Leytem⁴, E. Young⁵, R. Hagevoort⁶, and D. Mul-linax⁷, ¹*USDA-ARS, State College, PA*, ²*Fairlife LLC, Madrid, NY*,

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The US dairy industry has set the goal of attaining greenhouse gas (GHG) neutrality by mid-century. Reaching this goal requires the implementation of multiple strategies for mitigating and offsetting GHG emissions. A large range of animal feeding, manure handling, cropping and energy saving strategies were evaluated alone and in combination to quantify their potential for reducing the farmgate life cycle carbon (C) footprint of milk production. Representative dairy farms in major dairy regions of the US were simulated without and with various mitigation strategies using the Integrated Farm System Model (IFSM). The benefit obtained was quantified as the change in milk C footprint with a given strategy or combination of strategies compared with that of the base farm. For plausible scenarios, milk C footprint reductions achieved with individual strategies ranged from 0.3% to 20%. Strategies with the most benefit included anaerobic digestion of manure, a covered manure storage using a flare for methane destruction, and the use of a feed additive for mitigating enteric methane. A combination of appropriate strategies provided an average reduction in the milk C footprint over all farms of 46%. Potential offsets that might be achieved on a few farms through soil carbon sequestration or use of food waste in anaerobic digestion provided additional reductions of 7% and 4.9%, respectively. With the use of less probable maximum mitigation assumptions, individual reductions in GHG emissions ranged from 0.3 to 32% with a combination of appropriate strategies averaging 70% over all farms. Considering alternative metrics for quantifying the global warming effects of methane as a short-lived gas in the atmosphere provided a possible short-term solution for demonstrating climate neutrality of US dairy farms. If implementation of mitigation strategies over the next several years reduced total methane emissions from all farms at a rate greater than 1% per year, metrics such as GWP* would predict no effect on global temperature rise. Following life cycle assessment procedures, mitigation strategies have the potential for providing large reductions in GHG emissions but achieving long-term GHG neutrality over all US dairy farms appears improbable.

Key Words: dairy farm, greenhouse gas (GHG) mitigation, Integrated Farm System Model (IFSM)

1150 Estimating enteric methane emissions in South Asian countries: A model-based approach using feeding trial data. S. Alam^{*1,4}, M. G. Hasan², A. Imran², M. A. Shuva³, I. Jahan², and M. A. Islam², ¹Department of Dairy and Poultry Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh, ²Department of Dairy Science, Bangladesh Agricultural University, Mymensingh, Bangladesh, ³Division of Ruminant Nutrition, Department of Animal Sciences, Faculty of Agriculture, University of Göttingen, Göttingen, Germany, ⁴Department of Human Geography, University of Göttingen, Göttingen, Germany.

Enteric methane (Ent_{CH₄}) emissions from cattle significantly contribute to greenhouse gas emissions in South Asia, particularly in India, Bangladesh, and Pakistan, where approximately 250 million cattle produce around 300 million tons of milk annually. Direct measurement of Ent_{CH₄}, though precise, is resource-intensive and impractical for wider use. As an alternative, mathematical models endorsed by the Intergovernmental Panel on Climate Change (IPCC) and other frameworks are used to estimate emissions based on feeding trial data. This study evaluates Ent_{CH₄} emission estimates from 6 models categorized by dry matter intake (DMI) and gross energy intake (GEI): IPCC_{DMI},

IPCC_{GEI} (<http://www.ipcc-nggip.iges.or.jp/public/2006gl/index.html>), Ribeiro_{DMI}, Ribeiro_{GEI}, Patra_{DMI}, and Patra_{GEI}. A dataset of 91 feeding trials, including 1,684 cattle across India (59 trials), Bangladesh (20 trials), and Pakistan (12 trials), was analyzed. The tropical livestock unit (TLU) averaged 1.40 ± 0.42 , with mean DMI, GEI, and milk yield recorded at 9.43 ± 4.11 kg/day, 109.75 ± 50.86 Mcal/day, and 9.43 ± 4.11 kg/day, respectively. Among lactating cattle, the IPCC_{DMI} model estimated significantly higher Ent_{CH₄} emissions (243 g/day/cattle) compared with Ribeiro_{DMI} (205 g/day/cattle) and Patra_{DMI} (202 g/day/cattle) ($P < 0.05$). For nonlactating cattle, IPCC_{DMI} estimated 124 g/day/cattle, exceeding Ribeiro_{DMI} and Patra_{DMI} by 10 g/day. The GEI-based models followed a similar trend, with IPCC_{GEI} estimating the highest emissions for lactating cattle (191 g/day/cattle), whereas nonlactating cattle exhibited relatively consistent emissions across models (99–102 g/day/cattle). Across countries, no significant differences ($P > 0.05$) in Ent_{CH₄} emissions (g/day/TLU) were observed for lactating or nonlactating cattle, except in the Ribeiro_{DMI} and Patra_{DMI} models for nonlactating cattle, where Bangladesh exhibited higher estimates than India ($P < 0.05$). The findings suggest that IPCC models tend to overestimate Ent_{CH₄} emissions and highlight discrepancies among model estimates. This underscores the importance of selecting regionally appropriate models and refining estimation methodologies based on feeding trial data to enhance their applicability across South Asian livestock production systems.

Key Words: enteric methane, feeding trial, model

1151 A mechanistic model predicts diurnal methane emissions in dairy cattle under methane mitigation. E. M. Pressman* and E. Kebreab, Department of Animal Science, University of California–Davis, Davis, CA.

Enteric methane (CH₄) production is influenced by multiple factors, including basal diet, fractional outflow rate, rumen pH, and fermentative cofactor dynamics. These factors also affect the efficacy of anti-methanogenic feed additives (AMFA). We developed a novel rumen mechanistic model to predict diurnal enteric gas emissions that incorporates multiple AMFA, thermodynamic control of VFA pathways, mechanistic control of outflow and pH, and an expanded representation of hydrogen sinks. This study aimed to evaluate model predictions of diurnal CH₄ production against independent in vivo data under different AMFA supplementation scenarios. Model predictions were evaluated against treatment group averages at 17 diurnal time points from 2 separate studies: one with dairy cattle receiving no AMFA supplementation (“base scenario”) and another with dairy cattle supplemented with bromoform (CHBr₃)-containing seaweed at 0.5% and 1% OM (“low” and “high CHBr₃ scenarios,” respectively). Predictions were also evaluated against treatment group averages at 8 time points from a study where dairy cattle were supplemented with 3NOP at 6 doses (40, 60, 80, 100, 150, and 200 mg/kg DM). Model performance was assessed using Lin’s concordance correlation coefficient (CCC) and root mean square prediction error (RMSPE; % of observed mean). The model showed the lowest prediction error under the 3NOP scenarios (RMSPE = 42%, 41%, 44%, 32%, 32%, and 33% for 40, 60, 80, 100, 150, and 200 mg/kg DM, respectively). Prediction error was lower under the base scenario (RMPSE = 47%; CCC = 0.25) compared with the low CHBr₃ scenario (RMPSE = 98%; CCC = 0.0011) or high CHBr₃ scenario (RMPSE = 95%; CCC = 0.023). Average RMSPE of CH₄ production over 6 diurnal time points was 36% in a previous, similar mechanistic model without AMFA. While the increased complexity of our model may have contributed to greater prediction error, its CH₄ predictions remain comparable to existing models while offering a more detailed representation of rumen dynamics. Further evaluation of other rumen

metabolites is necessary to ensure the model's applicability in assessing the impact of AMFA on fermentation.

Key Words: methane, modeling, diurnal

1152 Heat stress increases enteric methane emissions yield and intensity while impairing rumen health and productivity in lactating dairy cattle. D. Onan-Martinez^{*1}, M. A. T. de Bari¹, H. Olmo¹, D. Vyas¹, J. Lance¹, I. M. Toledo¹, J. M. Tricarico², and G. E. Dahl¹, ¹Department of Animal Sciences, University of Florida, Gainesville, FL, ²Dairy Management Inc., Rosemont, IL.

Greenhouse gases (GHG) in the atmosphere are contributing to rising global temperatures. While livestock are a source of enteric methane (CH₄) emissions, they are also vulnerable to the adverse effects of climate change. Heat stress (HS) on dairy cattle has detrimental effects on productivity but the relationship between HS and CH₄ emissions remains unexplored. Therefore, we hypothesize that acute HS would increase CH₄ intensity in lactating dairy cattle. Forty lactating cows (~173 ± 23 DIM) were enrolled into one of 2 treatments for a 4-wk study in a randomized complete block design with lactation number and PTA genetic merit as blocking factors. Twenty cows received HS abatement with an evaporative cooling system (CL), while the remaining 20 cows received no abatement for HS in a neighboring pen. All cows were housed in a freestall barn equipped with Calan gates to collect daily individual dry matter intake (DMI) and were milked 3×/d. A GreenFeed system was used to measure enteric gas output and SmaXtec ruminal boluses were used to measure rumen temperature, ruminal pH and rumination time. The average environmental temperature-humidity index (THI) in the barns during the study was 79.2. Heat stress decreased DMI (18.9 ± 0.2 vs. 25.3 ± 0.2 kg/d SEM; $P < 0.01$) and energy-corrected milk (30.6 ± 0.5 vs. 42.6 ± 0.5 kg/d SEM; $P < 0.01$). Daily CH₄ emissions were reduced (326.7 ± 6.9 vs. 403.5 ± 8.3 g/d SEM; $P < 0.01$), whereas CH₄ yield (17.3 ± 0.3 vs. 15.8 ± 0.4, g/kg of DMI SEM; $P < 0.01$) and intensity (11.1 ± 0.3 vs. 9.0 ± 0.3 g/kg of ECM SEM; $P < 0.01$) increased due to HS. Furthermore, ruminal temperature was increased (40.7 ± 0.1 vs. 39.8 ± 0.1°C SEM; $P < 0.01$) while rumination time was decreased (432.2 ± 7.8 vs. 11.4 ± 6.7 min/d SEM; $P < 0.01$) by HS yet ruminal pH remained similar between treatments (6.37 ± 0.06 vs. 6.27 ± 0.06 SEM; $P = 0.27$). Our results indicate that HS increases CH₄ yield and intensity and disrupts rumen health providing further evidence of the negative effects of HS on the environmental impact and production efficiency of dairy cattle.

Key Words: global warming, enteric methane, rumen health

1153 Feeding strategies for cattle performance and greenhouse gases mitigation across the world. A. Ayemele Gnetegha^{*} and H. Jianlin, *Yazhouwan National Laboratory, Sanya, Hainan, China.*

Studies on effects of feeding strategies on cattle greenhouse gases emission are fragmented and cannot provide a global comparison of the feeding management practices that lead to an optimal production while substantially reducing methane and ammonia emissions. The present study aimed to explain the variability in enteric methane and ammonia mitigations and cattle milk performance due to several diet composition and structure, feed management, breeding, animal experimental designs and methane measurement tools. Effects size of 80 treatments were meta-analyzed from 4,394 screened papers that retained 31 articles distributed across 15 countries of 4 continents. Minerals were the feed additive type that increased milk yield the most (90%) and concomitantly, reduced methane production by 33% ($P < 0.05$). Feed additive dose that also

contributed to increase milk yield by 47% while reducing methane production by 58% ranged between 1% and 4% ($P < 0.05$); meanwhile, the impact of 3-NOP on health safety is still controversial. Although the partial mixed ration system highly contributed to milk yield (102%, $P < 0.05$), it did not contribute to methane yield reduction as much as the total mixed ration (39%). The highest methane yield reduction levels ($P < 0.05$) were recorded with GreenFeed (80%) and rotative experimental design (37%). Holstein breed was more productive with an increase of 46% of milk production whereas reducing methane production by 34% ($P < 0.05$). Grass forage increased ammonia by 5% while crop forage reduced it by 21% ($P < 0.05$). This meta-analysis shed light on minerals as feed additive group at dose range 1%–4% that can effectively contribute to achieve the climate neutrality goals while addressing the challenges of food security.

Key Words: ammonia, methane, milk yield

1154 Methane and carbon dioxide emissions of crossbreds of Holstein with Jersey, Montbéliarde, Normande, and Viking Red compared to purebred Holsteins. B. J. Heins^{*}, I. W. Haagen, and I. J. Salfer, *University of Minnesota, St. Paul, MN.*

The objective of this study was to compare methane emissions of Holstein (HO) and crossbred cows. Holstein and crossbred dairy cow data were from 2 herds, a grazing and a low-input conventional that were managed similarly at the University of Minnesota West Central Research and Outreach Center, Morris, Minnesota. Holstein cows ($n = 31$) were compared with GrazeCross crossbred cows ($n = 77$) composed of the Normande, Jersey, and Viking Red breeds and ProCross crossbred cows ($n = 148$) composed of the Montbéliarde, Viking Red, and HO breeds utilizing 52,568 emissions records. Data were collected from July 2023 to December 2024. Exhaled gas was collected and analyzed for methane and CO₂ with a GreenFeed system (C-Lock Inc., Rapid City, SD). Methane intensity was methane divided by milk production and methane yield was methane divided by dry matter intake. Independent variables for statistical analysis were fixed effects of herd (confinement vs. grazing), genetic group (HO vs. GrazeCross vs. ProCross), lactation number (primiparous vs. multiparous), hour of day of methane measurement, and sire breed group nested within genetic group. Methane emissions in the confinement system (393 g/d) were higher ($P < 0.05$) than in the grazing system (324 g/d) throughout the day. The GrazeCross (334 g/d) cows emitted less ($P < 0.05$) methane than ProCross (364 g/d) and Holstein (378 g/d) cows. Furthermore, the GrazeCross cows (11,696 g/d) had lower ($P < 0.05$) carbon dioxide emissions compared with ProCross (12,776 g/d) and Holstein (12,917 g/d) cows. Oxygen and Hydrogen emissions followed similar patterns for genetic groups. Methane intensity (methane/kg milk) was similar ($P > 0.22$) for Holstein (27.4) and GrazeCross (24.1) cows; however, ProCross (21.5) cows were lower ($P < 0.05$) compared with Holstein cows. ProCross cows (16.2) and GrazeCross (16.1) cows produced less ($P < 0.01$) methane per kg of DMI than Holstein (19.5) cows. Results suggest that crossbred cows have some benefits for reduced methane emissions in dairy systems.

Key Words: crossbreeding, pasture, methane

1155 Comparison of methods to measure enteric methane emissions in lactating dairy cattle. D. C. Reyes^{*1}, N. Seneviratne¹, P. Niu¹, V. Ramos¹, S. Pokharel¹, D. Carnovale², A. Sweeting², and J. W. McFadden¹, ¹Cornell University, Ithaca, NY, ²AgScent, Carwoola, NSW, Australia.

Our objective was to evaluate the GreenFeed (GF) and Agscent × Optiweigh (AO) systems by comparing spot gas measurements with respiration chambers (RC). Twelve late-lactation, pregnant Holstein cows (202 ± 12 d in milk, 46 ± 6.53 kg milk/d, and 710 ± 30 kg BW) were grouped by prior CH₄ emissions recorded using GF. Each group ($n = 3$) completed 3 9-d repetitions, with gas emissions measured for 3 d using GF, AO, and RC. Before data collection, cows were trained to use all systems. The GF and AO units remained stationary, with measurements ($n = 8$) taken every 9 h for representative 24-h sampling. The study was conducted under controlled conditions ($18 \pm 0.5^\circ\text{C}$; $57 \pm 7.4\%$ relative humidity). Cows were offered a TMR (DM basis: 55% corn silage, 12% grass haylage, and 33% concentrates) with ad libitum feed and water access. Dry matter intake and milk yield were recorded daily. Data were analyzed using PROC MIXED in SAS, and correlation coefficients were calculated in R. Dry matter intake and milk yield (mean = 21.5 and 33.3 kg/d) did not differ across methods. Methane emissions were higher ($P < 0.01$) in RC than in GF or AO (546 vs. 402 and 195 g/day). Consequently, CH₄ yield and intensity were greater ($P < 0.01$) in RC (22.8 vs. 16.5 and 8.0 g CH₄/kg DMI; 17.0 vs. 12.7 and 6.21 g CH₄/kg of milk). Emissions of CO₂ were also higher ($P < 0.01$) in RC relative to GF and AO (15.6 vs. 13.3 and 14.9 kg/d, respectively). The correlation between CH₄ emissions measured by RC and AO was -10% for whole-day emissions but improved to 41% when aligned to specific time points. A similar trend was observed for CO₂, with correlations increasing from -15% to 23% . In contrast, CH₄ and CO₂ correlations between RC and GF were higher for whole-day emissions (38% and 40%) but decreased to 27% and 21% when adjusted for specific times. Lin's concordance correlation coefficient indicated poor agreement for CH₄ emissions, with values of 0.09 (95% CI: 0.006, 0.174) for GF and 0.06 (95% CI: 0.049, 0.072) for AO. Our findings suggest that gas emissions from RC, GF, and AO lack sufficient agreement for interchangeability, requiring calibration factors or methodological adjustments.

Key Words: headbox system, respiration chamber, dairy cow

1156 Methane emission measurement via head chambers impacts dairy cattle performance in days following measurement events. C. McCabe*, E. Ross, Y. Pan, Y. Zhao, and F. Mitloehner, *University of California–Davis, Davis, CA*.

Respiration chambers and ventilated head chambers (HC) are considered the gold standard of methane (CH₄) emission measurements as their continuous samples capture the diurnal cycle of gas emissions. However, their negative impacts on normal cow behavior have been increasingly scrutinized. Two experiments evaluated separate rumen modifiers from October–December 2018 and October 2022–January 2023. In both experiments, cows were milked and fed twice per day at approximately 0600 and 1800 h and trained to use the HC before the experiment start. During experiment 1, CH₄ emissions from dairy cows were sampled via HC between milkings at 0600 and 1800 h and spent the overnight period in the freestall pen. In experiment 2, dairy cows were sampled via the HC at 0700–0900, 1300–1500, 1900–2100, and 0100–0300 h. Disruptions to dairy cows' normal daily routine were assessed via milk yield (MY) and dry matter intake (DMI) on the day of and each of the 3 d following a HC measurement. In experiment 1, DMI was lower on d 1 compared with the HC day (24.6 vs. 25.7 kg/d; $P < 0.05$), whereas in experiment 2, there was no difference in DMI between the HC day and any of the days following a HC measurement ($P > 0.05$). In experiment

1, there was a tendency for reduced MY on d 2 compared with the HC day (35.4 vs. 34.4 kg/d; $P = 0.06$), while in experiment 2, there was a tendency for reduced MY on Day 1 following a HC day (38.3 vs. 37.5 kg/d; $P = 0.09$). In both experiments, MY rebounded to previously observed levels by d 3. In both experiments, diurnal variation in CH₄ emissions was observed. In experiment 1, CH₄ emissions were greater from 1300 to 1900 h than from 0730 to 1300 h (24.1 vs. 22.4 g CH₄/h; $P < 0.05$), whereas in experiment 2, CH₄ emissions were greater from 1900 to 2100 h than from 0100 to 0300 h (15.9 vs. 14.1 g CH₄/h; $P < 0.05$). The timing and frequency at which emissions from dairy cows are sampled via HC impacts performance in days following sampling, demonstrating tradeoffs associated with this measurement tool.

Key Words: cow behavior, methane, methodology

1157 Location of sniffer in the automated milking system affects the methane concentration measurements and the inferred diurnal pattern. X. Ma*, S. E. Räisänen^{1,3}, M. Harrison², S. Zimmerman², and M. Niu¹, ¹*Department of Environmental Systems Science, Institute of Agricultural Sciences, ETH Zürich, Zürich, Switzerland*, ²*C-Lock Incorporated, Rapid City, SD*, ³*Department Animal and Veterinary Sciences, ANIVET Ruminant Nutrition, Aarhus University, Tjele, Aarhus, Denmark*.

The sniffer technique measures local gas concentrations by continuously drawing air through an intake system near the animal's muzzle and has been used for breeding low-emitting animals. The objective of this project was to evaluate the variations of CH₄ concentration measurements between sniffers placed in the automated milking system (AMS). We hypothesized that the position of the sniffer intake would influence average CH₄ concentration measurements and the inferred diurnal patterns. Ten sniffer units (SimpleScan, C-Lock Inc.) were used: 9 were arranged in a 3×3 grid (15 cm apart) over the AMS feed trough, and one was mounted above the AMS for background measurement. Methane concentrations were recorded in ppm (1-s basis; 1 L/min airflow) and averaged at each milking visit. Data were collected over 6 mo from 104 lactating dairy cows, resulting in 169,459 sniffer readings. All data were corrected for background. According to the cow-visit level data, the lower-middle sniffer recorded the highest CH₄ concentration (569 ± 157.9 ppm), followed by the centered sniffer (489 ± 248.8 ppm), whereas the top left sniffer recorded the lowest values (197 ± 115.6 ppm). The between-cow variability ranged from 44.9% to 62.3%. The cow-visit level data were further analyzed using a generalized additive mixed model, with sniffer nozzle position, breed, and parity as fixed effects, and cow as random effect. The smooth terms include Julian day to model the seasonality, days in milk by parity (interaction), and time of the day by sniffer position (interaction). A significant interaction between hour of the day and sniffer position was observed ($P < 0.01$), indicating that different sniffer positions exhibit distinct diurnal variations. In conclusion, the location of sniffer placed in the AMS affects both average and variability of the CH₄ concentration measurements, as well as the inferred daily patterns. In this study, a GreenFeed system was also used to measure the CH₄ fluxes of the same cows, and we are comparing the ranking coefficients and the repeatability of sniffers using GreenFeed data as a benchmark.

Key Words: methane, sniffer, diurnal pattern

Reproduction 1

1158 Combining early lactation automated estrus alerts and predictors of reproductive performance for grouping cows based on first service reproductive potential. A. L. Laplacette*, C. Rial, and J. O. Giordano, *Department of Animal Science, Cornell University, Ithaca, NY.*

The objective was to compare differences in first service (FS) pregnancies per AI (P/AI) for cows grouped based on the combination of data for reproductive performance predictors and automated estrus alerts (AEA) data collected before (BSy) and during (DSy) synchronization of ovulation. Data used were from primiparous (PP n = 569) and multiparous (MP n = 1,437) Holstein cows fitted with neck sensors (SenseHub, Merck) submitted to FS with GnRH (G), PGF (P), and progesterone (P4) based synchronization of ovulation programs that promote expression of estrus (G-7d-P-3d-G-7d-G-7d-P-1d-P-80h of AI in estrus-G-16h-TAI; G-7d-P-3d-G-7d-G-7d-P-1d-P-7d of AI in estrus-G+P4in-7d-P+P4out-1d-P-56h-G-16h-TAI). Health disorders, total milk yield (MY), genomic daughter pregnancy rate (GDPR), and AEA data were collected for the BSy (PP 0–67 DIM; MP 0–32 DIM) and DSy periods (PP 68–91 DIM; MP 33–56 DIM). Cows were grouped in high (H), medium (M), or low (L) GDPR and MY groups during the BSy and DSy period based on tertiles within parity. Cows with high MY, ≥ 1 health disorder, or low GDPR for the BSy (65%) or DSy (49%) period were considered to have risk factors (RF) for poor P/AI and 57% and 43% of cows had AEA during the BSy and DSy period. Combining levels of AEA and RF during BSy, cows were assigned to the AEA-NoRF (21%), NoAEA-RF (29%), NoAEA-NoRF (14%), and AEA-RF (36%) group. For DSy, cows were assigned to the AEA-NoRF (22%), NoAEA-RF (28%), NoAEA-NoRF (28%), and AEA-RF (21%) group. Logistic regression controlling for parity was used to compare P/AI. The largest differences in P/AI were between AEA-NoRF and NoAEA-RF (BSy 65% vs. 43%; DSy 63% vs. 47%). Also, P/AI was greater for AEA-NoRF than the remaining cohort (RC) of cows (all cows minus cows in AEA-NoRF) during BSy (AEA-NoRF 64%; RC 52%) and DSy (AEA-NoRF 63%; RC 52%). Fewer P/AI were observed for NoAEA-RF than the RC (i.e., all cows minus cows in NoAEA-RF) during BSy (NoAEA-RF 42%; RC 58%) and DSy (NoAEA-RF 47%; RC 57%). Combining data for AEA with other reproductive performance predictors before and during synchronization of ovulation could identify groups of cows with differences in P/AI to FS.

Key Words: automated estrus, predictor, fertility

1159 Predicting first service pregnancy outcome in lactating dairy cows with machine learning algorithms trained on behavioral, performance, and cow features data. A. L. Laplacette* and J. O. Giordano, *Department of Animal Science, Cornell University, Ithaca, NY.*

The objective was to evaluate the ability to predict first service (FS) outcomes with supervised machine learning algorithms (MLA) trained on a combination of behavioral and performance parameters and cow features data. Data were available from 569 primiparous (L1) and 1,437 multiparous (L2+) Holsteins submitted to FS with fertility programs that promote estrus. Cows were eligible for AI after 57 (L2+) or 92 DIM (L1). Behavioral and performance parameters predictors included daily milk yield, physical activity, and rumination time from calving to FS summarized within parity. The average, accumulated, CV, minimum, maximum and range for each parameter was summarized as a single

value (SP, single period dataset) and for periods of 5 to 46 d from calving to FS (MP, multiple periods dataset) to better represent patterns overtime. Previous lactation cow features data included lactation length, number of AIs, and milk, protein, and fat yield. Current lactation data included calving features, parity, genomic daughter pregnancy rate, AI season, semen type, health events, estrus events, and milk yield. Decision trees, Support Vector Machine, Logistic Regression (LR), and Extreme Gradient Boosting (XGB) models were trained to predict FS pregnancies per AI (P/AI) in all cows (52.0%), and L1 (59.3%) and L2+ cows only (48.9%). Models were trained using all (ALL) or a subset of predictors selected by domain expertise (DE). Training and testing datasets (80:20) were used to build models and evaluate performance. The 2 best MLA for all cows were XGB ALL with the SP (Se 49%, Sp 66%, Acc 59%), and the LR DE with the MP dataset (Se 71%, Sp 46%, Acc 59%). For L1 cows, the best 2 models were LR DE (Se 81%, Sp 39%, Acc 62%) and ALL (Se 78%, Sp 41%, Acc 61%) with the MP dataset, whereas for L2+ cows XGB DE with the SP (Se 53%, Sp 61%, Acc 57%) and LR DE with the MP (Se 53%, Sp 57%, Acc 55%) datasets were best. Machine learning algorithms trained on behavioral and performance parameters and cow features data did not perform well in predicting FS outcomes for lactating cows on a commercial farm.

Key Words: machine learning, artificial insemination (AI), performance

1160 Risk factors for pregnancy at first service in Holstein dairy herds using automated activity monitors. L. Campora*, E. Morrison, E. Ribeiro, R. Couto Serrenho, and S. J. LeBlanc, *University of Guelph, Canada.*

This longitudinal study evaluated the association between body condition score (BCS) change and pregnancy at first artificial insemination (P/1stAI), accounting for postpartum health conditions. Holstein cows (n = 1,818, 115 ± 8 cows/herd) from 19 herds in Ontario using automated activity monitors as the primary reproduction management tool were enrolled after calving. Body condition score change (Δ BCS) between wk 1 and 8 was categorized into ≤ -0.5 , -0.25 , or ≥ 0 . Purulent vaginal discharge (PVD; $>50\%$ pus) was diagnosed at wk 4, 6, and 8, whereas endometritis (ENDO; $>5\%$ PMN in uterine cytology) was diagnosed at wk 4, forming a 4-level variable based on combinations of PVD at any time and ENDO. Hyperketonemia (HYK) was defined as blood BHB ≥ 1.2 mmol/L at wk 1 or 2. Retained placenta (RP) was diagnosed when present >24 h after calving. A multivariable logistic regression model of P/1stAI with herd as a random effect was built in R, retaining variables with $P \leq 0.10$ and the main variable Δ BCS. The LSM (and their 95% CI) are reported. Overall, P/1stAI was 40% (38%–43%). Parity, RP, HYK, and uterine health were associated with P/1stAI, while Δ BCS was not. First-lactation cows were more likely to be pregnant at 1st AI (48%, 44%–52%), than second parity (39%, 34%–44%) or ≥ 3 rd parity cows (36%, 32%–39%). Non-RP cows were more likely to be pregnant to 1st AI (41%; 38%–44%) than cows with RP (30%, 21%–41%). Non-HYK cows had higher P/1stAI (41%, 38%–44%) than HYK cows (36%, 30%–41%). The probability of P/1stAI was 42% (39%–46%) for Non-PVD-ENDO, 32% (27%–38%) for BOTH, 44% (39%–50%) for ENDO only and 38% (33%–45%) for PVD only. Accounting for other variables, Δ BCS was not associated with P/1stAI. For cows with Δ BCS ≥ 0 (20% of cows) P/1stAI was 44% (39%–50%), for those that lost 0.25 (37% of cows) it was 39% (35%–43%) and for those that lost ≥ 0.5 (43% of cows) P/1stAI was 40% (36%–43%). These results suggest

that other reproductive and metabolic conditions should be accounted for in assessing the association of BCS change with P/1st AI.

Key Words: health, fertility, endometritis

1161 Effect of age at first timed AI in fertility outcomes of the first and second insemination. E. Karakaya Bilen¹, F. Eski¹, J. P. N. Andrade^{*2}, and A. Gümen³, ¹University of Cukurova, Adana, Turkey; ²West Texas A&M University, TX, ³University of Bursa Uludag, Bursa, Turkey.

This observational retrospective cohort study aimed to evaluate the effect of age at first timed artificial insemination (AI) on fertility outcomes of heifers (Holstein $n = 633$; Simental $n = 687$). Heifers (BW >320 kg and wither height >127 cm) were randomly assigned to one of 4 modified 6-d CoSynch protocols (d-10, PGF2 α ; d-8, GnRH + PRID; d-2, PGF2 α remove P4 implant (PRID); d-1 second PGF2 α ; d0, GnRH + TAI). Modifications were (1) an additional PGF2 α administration either 1 d before (P-Synch5/6) or 1 d after (P-Synch6/7) PRID removal, and (2) varying the timing of AI to either 48 h (with GnRH) or 56 h (8 h after GnRH) following PRID removal. After the first TAI, all heifers were resynchronized using PRID from d 12 to d 19 (ReBreed21). Early pregnancy diagnoses were made on d 21 based on corpus luteum (CL) blood flow using Doppler ultrasound ($<25\%$ of total CL area without blood flow assumed as nonpregnant while $\geq 25\%$ potential pregnant). Non-pregnant heifers with a follicle >10 mm diameter were immediately inseminated and given 100 μ g of GnRH on d 21. Age at first insemination (AFI) was categorized in tercile: Younger (AFI_1; $n = 451$; 13.0 ± 0.03 Min = 10.1–Max = 13.8), Median (AFI_2; $n = 442$; 14.6 ± 0.02 ; Min = 13.9–Max = 15.4), and Older (AFI_3; $n = 427$; 16.4 ± 0.03 Min = 15.5–Max = 19.4). Pregnancy per AI (P/AI) was determined on d 33 and d 63 after TAI. Data were analyzed with a generalized linear model including protocols, date of first TAI, breed, and age. Pairwise comparisons among age terciles were performed using Tukey's test. From the parameters included to enrich the model, only protocol affected P/AI D33 ($P < 0.01$) and P/AI D63 ($P < 0.01$) for first TAI. There was a tendency ($P = 0.06$) of lower P/AI in younger compared with medium age and older in the first AI (AFI_1 = 42.1%; AFI_2 = 48.4%; AFI_3 = 48.9%). Nevertheless pregnancy loss was lower ($P = 0.02$) in AFI_1 (1.0%) than AFI_2 (4.0%) or AFI_3 (4.4%). There was no effect of age in the fertility outcomes of the ReBreed21. In conclusion, optimizing the age of the first insemination could enhance reproductive success and reduce the need for multiple inseminations. This study is supported by the Turkish Academy of Science (TUBITAK, TOVAG 223O469).

Key Words: heifer, timed artificial insemination, fertility

1162 Rumination behavior changes the association between hyperketonemia and reproductive performance in dairy cows. C. Florentino*, A. Ravelo, F. Pena-Mosca, D. Moreira, M. Ruch, E. Shepley, and L. Caixeta, University of Minnesota, Saint Paul, MN.

In early lactation, dairy cows undergo metabolic shifts that elevate blood ketone levels, leading to hyperketonemia (HYK). While its impact on reproduction is known, the role of rumination behavior remains unclear. This study evaluated whether rumination recovery in the first postpartum week modifies the HYK-reproduction relationship. Cows from 2 Minnesota herds using timed artificial insemination were enrolled. Blood β -hydroxybutyrate was measured once between 2 and 9 DIM (5 ± 2), with a 1.2 mmol/L threshold classifying cows as HYK+ ($n = 108$) or HYK- ($n = 824$). Rumination behavior was monitored in the first postpartum week, and cows were retrospectively grouped based on daily

rumination change (min) from 2 to 8 DIM. The top 25% were high-speed (HS; 42.0 ± 17.2 min/d, HYK+ = 21; HYK- = 211), the middle 50% medium-speed (MS; 23.2 ± 7.4 min/d, HYK+ = 49; HYK- = 414), and the bottom 25% low-speed (LS; 1.6 ± 11.3 min/d, HYK+ = 38; HYK- = 199). Logistic regression models assessed pregnancy at first service and 150 DIM, whereas a Cox proportional hazard model evaluated time to pregnancy within 150 DIM. Models included RRG, HYK status, parity, and their interactions. When comparing LS HYK+ (LSM: 38%) to HS HYK+ (LSM: 50%), the risk of pregnancy at first service of the latter was 15% higher (RR: 1.15; 95% CI: 0.3 to 2.3). A smaller effect was found when comparing LS HYK- (66%) to HS HYK- (72%), where cows in the HS had 11% higher risk of pregnancy (RR: 1.11; 95% CI: 0.9 to 1.33). Comparing the risk of pregnancy at 150 DIM between LS HYK+ (60%) to HS HYK+ (81%), the HS group had a 47% higher risk of being pregnant at 150 DIM (RR: 1.47; 95% CI: 0.9 to 1.7). A smaller difference was found when comparing HYK- cows (LS: 75%; HS: 82%) where the risk of the HS group was 12% higher (RR: 1.12; 95% CI: 0.96 to 1.24). The hazard of pregnancy within 150 DIM when comparing LS HYK+ to HS HYK+ cows is 44% higher for the HS HYK+ cows (HR: 1.44; 95% CI: 0.7 to 2.9). A greater rumination recovery rate in the first week postpartum was associated with improved reproductive performance in HYK+ cows.

Key Words: ketosis, daily behavior, reproduction

1163 Optimal time of diagnosis of purulent vaginal discharge to predict pregnancy by 120 days in milk. C. F. Roeschmann^{*1}, E. Morrison¹, E. S. Ribeiro², R. Couto Serrenho¹, and S. J. LeBlanc¹, ¹Department of Population Medicine, University of Guelph, Guelph, ON, Canada, ²Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.

This prospective observational study aimed to evaluate the optimal time for diagnoses of purulent vaginal discharge (PVD) to predict pregnancy by the economic target of 120 d in milk (DIM). We used data from 531 Holstein cows from 4 dairy herds in Ontario. Dystocia, twins, stillbirth, milk fever, retained placenta, and metritis were recorded and combined into a 2-level variable. Calving and pregnancy dates were obtained from farm records. The diagnosis of PVD was conducted assessing the vaginal discharge (VD) at 25, 37, and 45 (± 3) DIM using a Metricheck device. Pregnancy by 120 DIM was evaluated using 5 mixed logistic regression models with different PVD classification approaches. For all models, PVD was defined as exudate containing $<50\%$ mucopurulent material (VD score = 2) or $\geq 50\%$ purulent material (VD score = 3). In the first 3 models, PVD was based on a single examination at either 21, 35, or 42 DIM, when 24%, 16%, and 13% of cows had PVD. The fourth model combined diagnoses at 37 and 45 DIM (22% positive for PVD), and the fifth model combined diagnoses at 25, 37, and 45 DIM (37% positive for PVD). All models accounted for the fixed effects of lactation, calving problems, early postpartum diseases, and the random effect of herd. The predictive ability of the models was assessed with ROC curves and the performance of the models was assessed by the area under the curve (AUC), sensitivity (Sn), and specificity (Sp). Data are presented as adjusted odds ratios (AOR) with 95% CI, and least squares means (LSM) $\pm$ SE as predicted probabilities. For cows that were pregnant by 120 DIM, the incidence of PVD was 23% at 25 DIM, 13% at 37 DIM, and 11% at 45 DIM. Cows with PVD at 45 DIM tended to have lower AOR of pregnancy by 120 DIM [49 ± 6 vs. $60 \pm 2\%$, AOR = 0.6 (0.4–1), $P < 0.1$, AUC = 0.61, Sn = 0.69, Sp = 0.49]. Cows with PVD at 37 or 45 DIM had lower AOR of pregnancy by 120 DIM [51 ± 5 vs. $62 \pm 2\%$, 0.6 (0.4–1), $P = 0.05$, AUC = 0.61, Sn = 0.66, Sp = 0.52]. Pregnancy by 120 DIM was not associated with PVD status at 25 DIM, or across

25, 37, and 45 DIM. These results indicate that PVD diagnosis closer to the end of the voluntary waiting period has greater predictive ability.

Key Words: Metricek, uterine disease, pregnancy

1164 Evaluation of ultrasonography for diagnosis of postpartum reproductive tract disease in dairy cows. R. Malet*, L. Cámpora, C. Roeschmann, E. Morrison, and S. J. LeBlanc, *University of Guelph, Guelph, ON, Canada*,

The objective of this study was to assess ultrasonographic criteria to diagnose postpartum purulent vaginal discharge (PVD; mucopurulent or worse vaginal discharge) or endometritis (ENDO; $\geq 6\%$ neutrophils in uterine cytology), using Metricek and cytology results, respectively, as reference tests, to provide a quicker, simpler on-farm diagnostic tool. Nine hundred seventy-nine Holstein cows (35% primiparous cows and 65% multiparous cows) were evaluated once at 22 to 28 DIM for PVD, received an ultrasonography exam (performed with an Ibox PRO/r ultrasound with a 7 MHz rectal linear transducer), and were sampled for cytology using a cytobrush adapted for use in cattle. The ultrasonography exam included taking record of ovarian structures and endometrial characteristics including cervix and uterine horns size, lumen shape, and the presence, quantity, and echogenicity of intraluminal fluid were evaluated based on literature. The prevalence of PVD and ENDO was 21% and 29%, respectively. Logistic regression models (GLIMMIX procedure) accounting for parity using mucopurulent vaginal discharge or worst, and PMN $\geq 6\%$, as the outcomes evaluated findings from ultrasonography for associations with PVD and ENDO, respectively. Predicted prevalence from the final multivariable models are presented. Distended lumen shape [>20 mm LSM = 47% (95% CI 36%–58%) vs. 18% (15%–22%)] and cervical diameter ≥ 4 cm [LSM = 30% (22%–38%) vs. 14% (9%–21%)] were associated with PVD. Presence of fluid [LSM = 38% (33%–43%) vs. 28% (23%–33%)] and cervical diameter [<4 cm LSM = 26% (19–34%); = 4 cm LSM = 34% (29%–39%); >4 cm LSM = 42% (33%–51%)] were associated with ENDO. Cows with larger volumes of fluid (≥ 20 mm) had greater odds of being diagnosed with ENDO [LSM = 47% (36%–58%) vs. 28% (23%–33%)] compared with those without intraluminal fluid. No ultrasonography parameters were associated with diagnosis of subclinical endometritis (i.e., ENDO without PVD). The maximum sensitivity and specificity of ultrasonography to diagnose PVD or ENDO were achieved when the tests were evaluated in parallel. If cows had either distended lumen shape or cervix ≥ 4 cm, the sensitivity was 37%, and specificity was 83% (positive predictive value [PPV] = 38%; negative predictive value [NPV] = 83%) for PVD. The presence of intraluminal fluid and/or cervix diameter ≥ 4 cm yielded 74% sensitivity, and 36% specificity (PPV = 37%, NPV = 73%) for ENDO. Ultrasonography could be used to help rule out PVD, but it is not accurate to diagnose PVD or endometritis.

Key Words: ultrasonography, purulent vaginal discharge (PVD), endometritis

1165 16S rRNA metagenetic insights into spatiotemporal shifts in the bovine reproductive tract microbiome. T. Raz*^{1,2}, S. Druker^{2,3}, and R. Sicsic², ¹Advanced Academic Programs, Krieger School of Arts and Sciences, Johns Hopkins University, Baltimore, MD, ²Koret School of Veterinary Medicine, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel, ³Hachaklait, Mutual Society for Veterinary Services, Caesarea, Israel.

Recent studies highlight the crucial role of reproductive tract microbial communities in shaping mammalian health and reproductive outcomes. The transition from late pregnancy to early postpartum, marked by cervical opening, triggers extensive contamination of the female reproductive tract. We and others have conducted several 16S-rRNA metagenetics studies on Holstein-Friesian cows, identifying bacteria undetectable by traditional methods. Our recent findings reveal distinct spatiotemporal microbial shifts between the prepartum vagina (258 ± 4 d gestation) and the postpartum vagina and uterus (7 ± 2 DIM) in first-pregnancy Holstein-Friesian heifers ($n = 33$). The prepartum vagina and postpartum uterus were most distinct, with the postpartum vagina showing intermediate profiles. A significant reduction in bacterial richness and diversity in the postpartum uterus suggests a selective niche shaped by parturition-related endocrine and physiological changes. Our findings indicate that vaginal microbiota may seed the postpartum uterine microbiome, with environmental influences further shaping its composition. At 5–12 DIM, uterine bacterial load did not differ between healthy and metritic cows ($n = 129$). However, metagenomics revealed that metritis cows harbored a distinct endometrial microbial profile dominated by Bacteroidetes and Fusobacteria, particularly *Bacteroides*, *Porphyromonas*, *Fusobacterium*, and *Tissierellaceae* spp. In contrast, healthy cows exhibited greater microbial diversity, though some displayed a “metritic-like” community, suggesting individual variability in immune responses, as supported by our RNAsq analysis. Moreover, our findings confirm that Koch’s postulates are not fully met in postpartum uterine diseases, supporting the need to move beyond pathogen-centric models and toward a broader understanding of host-microbe interactions in uterine health. Future studies integrating advanced bioinformatics and refined disease definitions will provide deeper insights into microbial dynamics and their impact on reproductive health, offering new opportunities for improved prevention, diagnosis, and treatment strategies.

Key Words: metagenetics, reproductive microbiome, metritis

1166 Differences in uterine and plasma metabolome associated with vaginal discharge characteristics in postpartum dairy cows. M. H. Rashid*¹, S. Casaro², R. S. Bisinotto², R. C. Chebel², J. E. P. Santos², C. D. Nelson², J. G. Prim³, S. J. Jeon⁴, R. C. Bicalho⁵, J. P. Driver⁶, K. N. Galvão², and C. C. Figueiredo¹, ¹Washington State University, Pullman, WA, ²University of Florida, Gainesville, FL, ³Auburn University, Auburn, AL, ⁴Long Island University, Brookville, NY, ⁵FERA Diagnostics and Biologicals, College Station, TX, ⁶University of Missouri–Columbia, Columbia, MO.

This study aimed to evaluate differences in plasma and uterine metabolome associated with vaginal discharge (VD) characteristics in lactating Holstein dairy cows. VD was visually evaluated on the day of calving (D0), 3, 7, and 10 DIM and scored as follows: VD1 = clear mucus/lochia, $n = 14$; VD2 = clear mucus with flecks of pus, $n = 18$; VD3 = mucopurulent with $<50\%$ of pus, $n = 13$; VD4 = mucopurulent with $\geq 50\%$ of pus or reddish/brownish mucous discharge not fetid, $n = 7$; VD5 = watery, reddish/brownish, and fetid discharge, $n = 52$. Cows were grouped based on the highest VD observed within 10 DIM which was considered day of diagnosis (Dx); none of the cows had a subsequent increase in VD after Dx. Plasma samples were retrieved from cows at 14 d prepartum (PP), D0, and Dx, while uterine contents were retrieved at D0 and Dx only via low volume lavage using sterile saline solution. Plasma and uterine metabolome were analyzed by untargeted gas chromatography-mass spectrometry. A total of 167 and 253 metabolites were annotated for plasma and uterine datasets, respectively. Differences in plasma

and uterine metabolome according to VD at each time interval were assessed via Principal Component Analysis and PERMANOVA, using parity (balanced across VD) as random effect. Pairwise PERMANOVA was used to identify the differences among VD groups. In plasma at PP, difference was observed only between VD1 and VD5 ($P < 0.01$). At D0, VD5 was different ($P < 0.01$) from VD1–3, but not from VD4; no differences were observed among VD1–4. At Dx, VD5 differed ($P < 0.02$) from all other VD groups; no differences were observed among VD1–4 ($P \geq 0.11$). In the uterus, no differences ($P > 0.05$) were associated with VD at D0. At Dx, VD5 differed ($P < 0.01$) from all other VD groups; no differences were observed among VD1–4 ($P \geq 0.29$). Uterine metabolites associated with bacterial function and inflammation including spermidine, erythritol, and arachidonic acid were more abundant in VD5 than in other VD groups. The changes in plasma and uterine metabolomic composition were mainly restricted to VD5 as compared with VD1–4, that were similar among each other.

Key Words: metritis, purulent vaginal discharge, lavage

1167 Genome-wide DNA methylation analysis of uterine endometrium in dairy cows with early postpartum metritis. M. A. Afzal*, S. K. Sivasankaran, M. O. Caldeira, I. Sellmer Ramos, and M. C. Lucy, *University of Missouri, Columbia, MO*.

Postpartum uterine disease has long-term detrimental effects on fertility. Our previous findings indicated that postpartum uterine disease alters the transcriptome of the uterine endometrium in mid-lactation cows. We hypothesized that these transcriptomic changes are driven by an epigenetic mechanism involving genome methylation. The objective was to assess genome methylation in the caruncular (CAR)

and intercaruncular (ICAR) endometrium of cows that were previously diagnosed with metritis or remained healthy. First-parity Holstein cows diagnosed with clinical metritis ($n = 6$) or identified as healthy ($n = 6$) were studied. Cows were assigned to slaughter at approximately 80 d postpartum ($n = 6$) or 165 d postpartum ($n = 6$). DNA was extracted from CAR and ICAR endometrium using DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA). A total of 24 DNA samples (6 metritis CAR, 6 metritis ICAR, 6 healthy CAR, and 6 healthy ICAR) were submitted to the MU Genomics Technology Core for whole-genome bisulfite sequencing (WGBS) generating 130–165 million paired-end reads per sample. Sequence quality was measured, and adapters and low-quality reads were removed. The processed reads were aligned to the *Bos taurus* reference genome (ARS-UCD1.3), using Bismark. Alignment yielded 60 to 72% uniquely mapped reads. These reads were subsequently used for DNA methylation calling in Bismark, followed by downstream analysis of differentially methylated sites (DMSs) and differentially methylated regions (DMRs) using MethylKit in R. Preliminary analyses, using hierarchical clustering, revealed 2 key findings. First, CAR and ICAR were clustered together, indicating that the 2 tissue types were highly similar. Second, no clear separation was observed between healthy and metritis cows across all samples, suggesting that global methylation was not affected by disease. However, these results do not rule out the possibility of differential methylation at specific CpG sites or genomic regions, which we will investigate through DMS and DMR analysis using an FDR threshold of $P < 0.05$. We aim to integrate methylome and transcriptome data to identify methylation differences that may drive transcriptomic changes linked to reduced fertility in cows with postpartum metritis.

Key Words: metritis, endometrium, methylome

Applied Nutrition Platform Session: Leveraging High Oleic Soybeans for Dairy Cow Profitability

1168 Critical knowledge to get the most from high oleic soybeans. L. Davis*, *Quality Roasting Inc., Valders, WI.*

Numerous studies published in the 1990s consistently showed that feeding dairy cows roasted soybeans compared with raw soybeans or soybean meal led to increased milk yield and fat-corrected milk. Heat treatment, including roasting, increases rumen undegradable protein (RUP) and particle size reduction of the whole roasted soybean improves digestibility in the small intestine. Subsequent research into the total rumen unsaturated fatty acid load (RUFAL) identified that diets high in RUFAL can disrupt ruminal fermentation and milk fat synthesis, causing practicing nutritionists to limit oilseeds in the ration. The introduction of high oleic soybean varieties that replace much of the polyunsaturated fatty acids with oleic acid, a monounsaturated fatty acid, reduces the risk of milk fat depression and has created new interest in feeding higher levels of roasted or expelled soybean products from high oleic soybeans. Optimal roasting methodology, including time and temperature, as well as steeping and cooling times have been refined. Further processing that includes extrusion and expelling can increase RUP levels beyond those achieved with roasting alone and can reduce the fat content of the finished product when that is warranted. Quality control measurements such as protein dispersibility index (PDI), Ross assay, or multi-step in vitro protein evaluation (MSPE) methods are tools that can help optimize the end product.

Key Words: high oleic soybean, roasting, expelling

1169 Effects of feeding a diet with high oleic soybean oil on milk and enteric methane production in lactating cows. B. Bae^{*1}, J. Kim^{1,2}, K. Park^{1,2}, H. Hu¹, M. L. Eastridge³, and C. Lee^{1,2}, ¹*Department of Animal Sciences, The Ohio State University, Wooster, OH*, ²*Interdisciplinary Nutrition Program, Department of Animal Sciences, The Ohio State University, Wooster, OH*, ³*Department of Animal Sciences, The Ohio State University, Columbus, OH.*

Feeding fat enriched with oleic acid showed positive effects on milk production in the literature. In various in vitro studies, oleic acid was effective in mitigating methane production. The objective of this study was to determine the effects of a diet with high oleic soybean oil on milk and enteric methane production. The study was conducted with 8 mid-lactation Holstein cows in a crossover design. Cows were randomly assigned to one of 2 treatments within each block: a diet supplemented with fatty acids (FA; 2% of dietary DM) enriched with saturated FA (28% C16:0, 55% C18:0, and 7% C18:1; CON), or the CON diet supplemented with high oleic soybean oil (8% C16:0, 4% C18:0, and 77% C18:1; HOSO) by replacing the saturated FA. Each period consisted of 14 d of diet adaptation and 7 d of sampling for lactation performance. We developed a facemask system and used to collect enteric gas samples for individual cows every 3 h in a 24 h cycle over 3 d. All data were analyzed using PROC MIXED of SAS, with period and cow within block as random effects and treatment as a fixed effect. Dry matter intake and BW were not affected by treatments. Milk yield was not affected but numerically greater (44.4 vs. 42.3 kg/d; $P = 0.13$) for HOSO compared with CON. Therefore, milk yield per DMI tended to be greater ($P = 0.09$) for HOSO. Milk protein and lactose yields did not differ between treatments. Milk fat concentration (3.92 vs. 4.48%) and yield (1.70 vs. 1.88 kg/d) were lower ($P < 0.01$) for HOSO com-

pared with CON. Although ECM did not differ, cows on HOSO had lower ECM per DMI (1.43 vs. 1.48; $P = 0.04$) compared with CON. No differences in enteric methane production (357 g/d) and methane production per DMI (11.6 g/kg) were found between HOSO and CON. In conclusion, feeding high oleic soybean oil may increase milk yield and feed efficiency, but decrease milk fat yield, suggesting that energy utilization will differ between feeding different types of FA. In addition, feeding high oleic soybean oil had no effect on methane production.

Key Words: gas facemask, milk fat, oleic acid

1170 Interaction of soyhulls and increasing fat with high oleic soybean products on milk production and fatty acid profile in dairy cows. M. Ibrahim^{*1,2}, P. Doane³, and K. J. Harvatine¹, ¹*Department of Animal Science, The Pennsylvania State University, University Park, PA*, ²*Faculty of Veterinary Medicine, Cairo University, Giza, Egypt*, ³*Ag Processing Inc., Omaha, NE.*

Supplementing fats in dairy diets is crucial to meet energy needs and maintain optimal milk fat yield in dairy cows. We hypothesized that increasing dietary fat from different high oleic soybean (HOSB) feeds in combination with increasing diet digestibility with soyhulls would increase milk fat yield. Eighteen multiparous Holstein cows (DIM = 135 $\pm$ 21) were used in an incomplete Latin square design with 21-d periods. Treatments were a 2 $\times$ 3 factorial of soyhulls and soybean sources. Soyhulls were fed at 10% of DM replacing corn silage and alfalfa haylage. Soybean sources were a mixture of normal and heat-treated solvent-extracted meal (CON), expeller HOSB meal (EXP), and roasted HOSB (RSB). The RSB were fed at 10% of DM and others were fed to supply equal CP and similar RUP. Data were analyzed in JMP Pro 18 and the model included the random effects of cow and period and the fixed effects of soyhulls, soybean source, and their interaction. There was no interaction between soyhulls and soybean type ($P > 0.05$). Soyhulls increased DMI (2.8 kg/d, $P < 0.001$) and milk (2.9 kg/d, $P < 0.001$) and milk protein (105 g/d, $P < 0.001$) yield and decreased milk fat (0.3 units, $P < 0.001$) compared with CON. Roasted HOSB decreased milk protein compared with CON (0.14 units; $P < 0.01$) but soybean source did not change DMI or milk and milk fat or protein yields ($P > 0.05$). The concentration of <16C FA in milk fat was decreased by both EXP and RSB ($P = 0.03$), although the yield of de novo FA was not changed ($P = 0.12$). The concentration of 16C FA was decreased in EXP and RSB compared with CON (26.3, 29.1, 32.4; $P < 0.001$). Yield of 16C FA was reduced 85 g/d in RSB ($P < 0.001$), but EXP was not different from CON. Concentration and yield of >16C FA in milk fat were increased 68 and 163 g/d in EXP and RSB compared with CON ($P < 0.001$). In conclusion, increasing dietary fat from HOSB increased milk preformed FA yield and decreased C16 FA and protein concentration, but had no effect on milk and milk fat yield and DMI. Soyhulls increased DMI, milk and milk protein yield and decreased milk fat concentration.

Key Words: high oleic soybean, soyhull, milk fat

1171 Integrating scientific insights into feeding high oleic soybeans to dairy cows. A. M. Bales^{*1,2} and A. L. Lock¹, ¹*Michigan State University, East Lansing, MI*, ²*Caledonia Farmers Elevator, Caledonia, MI.*

High oleic acid soybeans (HOSB) are a feed ingredient that hold promise for improving the performance of dairy cows. This is due to their high oleic acid (OA) content, a fatty acid (FA) we have previously demonstrated to increase FA digestibility and milk production responses in high-producing dairy cows. The unique OA content (75%–80% total FA) of HOSB makes it an attractive alternative to conventional soybeans, cottonseed, and some FA supplements. Notably, HOSB can be produced regionally in the USA by dairy and soybean producers, making it an attractive option. With the recent publication of research evaluating HOSB, there has been significant growth in the use of HOSB in lactating dairy cow diets. In this review we will provide a summary of recent research with HOSB and share examples of on-farm impacts of feeding HOSB. These industry examples will discuss how to utilize HOSB as a protein and FA-rich feed ingredient, decisions regarding diet formulation with HOSB, and processing methods of HOSB. Our previous research has demonstrated that feeding roasted and ground HOSB into the diet can enhance milk and milk component yields when compared to both a non-FA supplemented control and raw HOSB diets. Recent research

investigated the potential benefits of incorporating specific dietary ingredients with HOSB to further increase milk production responses. These studies demonstrated that both dietary acetate and supplemental palmitic acid could improve milk fat yield with HOSB; as well as emphasizing that production responses to different dietary 18-carbon FA (stearic vs. OA) will vary. Additionally, we examined the impact of high oleic expeller meal and the potential of this product as a quality protein source with a reduced supply of OA. The outcomes of these studies provide valuable insights into the potential and limitations of HOSB feeding, enabling dairy farmers and nutritionists to make informed decisions regarding its optimal dietary utilization. The challenge and opportunities will be to effectively apply this knowledge in feeding and management of today's high-producing dairy cows. Further research will undoubtedly continue to develop and improve our understanding of this unique opportunity with OA that HOSB offers for optimizing milk production responses in a profitable and sustainable manner.

Key Words: oleic acid, soybean, milk fat

Ruminant Nutrition 1: Calves and Heifers

1172 Growth performance of neonatal calves fed milk replacer 2 versus 3 times per day. D. P. Casper^{*1,2}, ¹Casper's Calf Ranch, LLC, Freeport, IL, ²North Carolina A&T University, Greensboro, NC.

Several calf studies demonstrate advantages when feeding greater protein and/or milk solids amounts. However, a prior experiment evaluating both feeding frequency (2X vs. 3X/d) and solids intake demonstrated no effects on growth performance during the first 14 d of the experiment. In addition, an aggressive weaning program reduced growth rates for calves fed 3X compared with calves fed 2X/d due to lower calf starter intakes. That study was repeated with minor changes to the feeding and weaning programs. Fifty 2- to 5-d-old Holstein bull calves housed in calf hutches were blocked by body weight (BW) and randomly assigned to 1 of 2 treatments. Treatments were (1) 2X: milk replacer (MR) fed 2x/d, and (2) 3X: MR fed 3x/d. The MR was fed at 15% solids at 0630 and 1800 h and the third (3X/d) feeding was at 1200 h. During d 1–14 calves fed both treatments were fed 0.567 kg/d MR in 2 equal feedings. Starting on d 15, MR amounts were increased to 0.85 kg/d for calves fed 2X, while calves fed 3X received a third equal MR feeding to provide 1.275 kg/d. To facilitate improved weaning transition for calves fed 3X/d they were reduced to 2X/d feeding during wk 6 and during wk 7; both 2X and 3X calves were reduced to 1x/d feeding with weaning occurring after d 49. Calves were weighed weekly with a 63-d experiment period. Calf starter (CS) was individually fed and orts were weighed daily with water offered free choice. Data were analyzed using PROC MIXED using a randomized complete block design having 2 treatments with week as a repeated measurement. Initial BW was a significant ($P < 0.01$) covariate and study average BW (63.5 and 65.8 kg for 2X and 3X, respectively) was numerically greater ($P < 0.11$) for calves fed 3X compared with calves fed 2X, but BW gains (42.2 and 44.6 kg) were similar ($P > 0.30$). A treatment by week interaction ($P < 0.04$) demonstrated improved BW for calves fed 3X during wk 4, 5, and 6 compared with calves fed 2X. Final BW (87.4 and 90.3 kg) tended to be greater ($P < 0.10$) for calves fed 3X compared with calves fed 2X. A treatment by week interaction ($P < 0.04$) demonstrated a reduction in CS intake for calves fed 3X during wk 5, 6, and 7 compared with calves fed 2X. Feed conversions (0.545 and 0.541 kg/kg) were similar for calves fed both treatments. Providing an additional MR feeding that increases both MR feeding volume and intake demonstrated minimal BW gains.

Key Words: calf, times feeding, growth performance

1173 Effect of fortifying fresh whole milk with a tributyrin-containing fat concentrate on growth, feed intake, and health in calves. J. N. Wilms*, J. Martin-Tereso, and L. N. Leal, *Trouw Nutrition R&D, Boxmeer, the Netherlands.*

Increasing the total solids (TS) by adding a fat concentrate to whole milk (WM), increases total nutrient supply to restricted-fed calves. Furthermore, a fat concentrate containing tributyrin may positively influence health, as multiple studies have reported improved fecal scores with tributyrin supplementation in liquid feeds. This study evaluated the effect of increasing TS in fresh WM using a functional spray-dried fat concentrate containing tributyrin on calf growth and health. Twenty-seven Holstein male calves (43.2 ± 2.51 kg, 25.3 ± 8.10 h of age) were blocked by BW and arrival sequence. Within each block, calves were randomly assigned to the following treatments (9 calves/group): (1) fresh WM (CON), (2) fresh WM with fat concentrate formulated with vegetable oils (FC), and (3) fresh WM with a fat concentrate formulated

with the same vegetable oils plus tributyrin (FC-T). Fat concentrates were supplemented at 35 g/L of WM resulting in 16% TS. Calves were individually housed and fed twice daily: 6 L/d from d 1–3, 7 L/d from d 4–9, and 8 L/d from d 10–42. Step-down weaning was induced from d 43–63: 6 L/d from d 43–49, 4 L/d from d 50–56, and 2 L (one meal) from d 57–63. From d 64–84, calves were fully weaned. Calves had ad libitum access to chopped straw and water from arrival, with starter feed provided from wk 2. Fecal consistency was evaluated daily after morning feeding using a binomial score. Body weight was recorded weekly. Continuous variables were analyzed using a mixed-effects model in SAS with the calf being the experimental unit. Discrete variables were analyzed using mixed-effects logistic regressions. At wk 3, FC-T calves had more diarrhea than CON ($P = 0.04$), while FC showed no difference. At wk 4, CON had more diarrhea than the other groups ($P < 0.01$). Milk intake did not differ ($P = 0.37$), except for the first 7 d, when calves fed CON had fewer refusals ($P = 0.04$). Starter feed intake did not differ ($P = 0.72$); however, in the last 14 d of rearing, intake was higher in the FC-T group than in the other groups ($P < 0.01$). The BW ($P = 0.15$) and ADG ($P = 0.21$) over the 12-wk period did not differ, but postweaning BW was lower in calves fed CON than other groups ($P = 0.05$). Increasing the TS in WM enhanced growth. The tributyrin-containing fat concentrate did not show improved health, but it increased postweaning starter feed intake.

Key Words: dairy calf, whole milk, butyric acid

1174 Effects of dietary resin acids supplementation on overall health and performance of Holstein milking calves. L. F. D. S. Caixeta¹, M. M. D. Vieira¹, K. L. Rodrigues¹, P. A. D. O. Machado¹, R. H. D. F. Rodrigues¹, J. V. Araujo¹, E. J. Bungenstab^{*2}, G. A. Gomes², and R. Nassar Ferreira¹, ¹Universidade Federal de Goias, Goiania, Goias, Brazil, ²AB Vista, Plantation, FL.

The objective of this study was to explore the benefits of feeding resin acids-based product (RA; containing 9% of resin acids) to Holstein crosses containing 1/4 *Bos indicus* (Zebu) bull calves and measure the benefits on overall health based on blood parameters, fecal microbiota, and growth. Calves ($n = 20$) with 20 ± 3 d of age, weighing on average 50.4 ± 7.2 kg, at d 1 of the study, were randomly assigned to control or RA treatments ($n = 10$ /treatment). Calves were fed milk twice a day at 3 and 2 kg/head in the morning and in the afternoon, respectively. The RA-fed calves received 2 mL/d of RA, 1 mL in the morning and 1 mL in the afternoon, mixed with the milk. Fresh water and hay were provided ad libitum throughout the experiment. A common starter feed was fed ad libitum in increasing amounts up to the limit of 600 g/head/d. Fecal collection was performed on d 17 and d 52 with *Escherichia coli* and multidrug-resistant *E. coli* analysis performed. Blood was sampled from all calves on d 50 to perform a comprehensive metabolic panel. The test lasted 61 d, and calves were euthanized at the end of the trial. Body weight (d 1, 38, 58) and blood (d 50) data were subjected to ANOVA analysis, whereas *E. coli* and multidrug-resistant *E. coli* presence (d 17 and 52) were analyzed via chi-squared, with differences deemed significant at $P \leq 0.05$ and $P \leq 0.10$ considered a tendency. Chi-squared analysis revealed that the frequency of multidrug-resistant *E. coli* was greater ($P \leq 0.05$) for control calves (45%) compared with those fed RA (5%). Twenty percent of the control calves tested negative for *E. coli* compared with 35% of calves who were fed RA. There was an increase ($P \leq 0.05$) in blood platelets for the calves on the control compared with the calves fed RA ($402,000$ vs. $306,000$ mm³), signaling a possibility of

infections or inflammatory disorders. The RA treatment had no effect on any other blood parameter measured. Therefore, RA did not affect the levels of liver enzymes (ALT, AST) and kidney function markers (BUN, creatinine), indicating no adverse effect. At 38 d calves receiving RA tended ($P = 0.08$) to be heavier (75.4 kg) than the calves on the control (64.1 kg). These results indicate that RA supplementation has potential to improve calf health and reduce the incidence of multidrug-resistant *E. coli*.

Key Words: immunity booster, microbiome modulation, overall health

1175 Assessing consistency in solids and temperature of milk replacers fed via automatic milk feeding devices. S. Silvestrelli^{*1}, A. Formigoni¹, and A. Bach^{2,3}, ¹Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy, ²Department of Animal Science, University of Lleida, Lleida, Spain, ³Institució de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

Use of automatic milk feeder (AMF) for calves housed in groups has progressively grown. Changes or inconsistencies in the DM of the milk replacer (MR) may cause digestive upsets in calves. The objective was to assess the intermeal variation in temperature and DM content of MR fed via AMF. For a 29-d period, 2 farms located in Catalonia (Spain), each equipped with 5 AMF with 4 teats, were visited 3 times per week to collect MR samples from each teat and AMF during 3 h. Farm A prepared MR at a target DM of 13.5%, whereas farm B targeted a 14% DM. Each AMF had 2 lines close to (180 cm) and 2 lines far from (300 cm) the mixing device. At each meal, temperature and DM (using an oven) of the MR at the teat of each line of the AMF were measured. Data were summarized by farm, AMF within farm, and line within AMF and average and coefficient of variation (CV) of the 302 observations. Data were split by farm, and analyzed using ANOVA including the effect of the AMF, length of teat line (i.e., close or far), and their 2-way interaction. In farm A, temperature of MR was similar across AMF, but it was colder in the teats far (31.6 ± 0.95°C) than in those close (35.0 ± 0.96°C) to the AMF. However, the CV of MR temperature differed between AMF, with values ranging from 10.0 to 28.2%, regardless of teat distance. In farm B, there were differences in temperature of MR between AMF, and the temperature CV differed among AMF (from 1.35 to 7.8%), regardless of the line length. In farm A, an AMF produced a MR with an average of 13.5 ± 0.30%, much greater than the one with the minimum average DM (11.9 ± 0.30%), and the CV of DM tended to differ between AMF, with the greatest CV being 8.8% and lowest 3.7%. In farm B, 2 AMF mixed a MR with a greater DM (11.9 ± 0.46%) than the others (8.44 ± 0.68%), but in both cases the dilution rate was well below the target of 14%. Distance from the AMF had no effect on average or CV of DM in the MR. It is concluded that DM content and MR temperature prepared by AMF varies between and within AMF over time. Furthermore, the origin of variation in temperature of MR is the machine, not the line assembly.

Key Words: facility, nourishing, variation

1176 Growth performance, metabolic, and hematological response of preweaning Fleckvieh dairy calves fed milk replacer or whole milk. M. Sfulcini^{*1}, L. Cattaneo¹, A. Minuti¹, J. J. Loores², E. Trevisi¹, C. Cavallo³, L. Liotta³, and V. Lopreato³, ¹Department of Animal Science, Food and Nutrition (DIANA), Faculty of Agricultural, Food and Environmental Sciences, Università Cattolica del Sacro Cuore, Piacenza, Italy, ²Mammalian NutriPhysioGenomics, Department of Animal Sciences and Division of Nutritional Sciences,

University of Illinois, Urbana, IL, ³Department of Veterinary Sciences, Università di Messina, Messina, Italy.

This study aimed to compare performance, metabolic, and hematological response of female Fleckvieh calves fed either whole milk (WM) or milk replacer (MR), supplying similar total solids, from birth to 60 d. After receiving 3 L of colostrum, calves were assigned to WM ($n = 9$; DM basis, 29.1% protein, 27.3% fat, and 37.3% lactose; 5.25 Mcal/kg of DM) or MR ($n = 9$; DM basis, 22.9% protein, 19.1% fat, and 51.73% lactose; 4.76 Mcal/kg DM; Elvor, Maen Roch, France). The MR was prepared at 130 g/L to match WM solids. Calves were housed in individual hutches, fed 3 L of milk twice daily until 53 d and then 3 L of milk once daily until weaning (60 d). Calves had *ad libitum* access to starter and intake was recorded daily. The BW was measured and blood samples were collected throughout the study. Data were analyzed using repeated measures mixed models (PROC GLIMMIX, SAS). Total DMI was similar between groups. Overall, WM calves had greater BW ($P = 0.01$), tended to have greater ADG (0.77 vs. 0.85 kg/d in MT and WM; $P = 0.07$), and had a greater gain efficiency (kg of BW gain/kg of DMI; $P < 0.01$) than MR. From 21 to 60 d, WM calves were 4 kg heavier than MR ($P < 0.01$). The WM had greater plasma glucose (5.6 vs. 6.2 ± 0.35 mmol/L in MR and WM; $P < 0.01$), urea (3.0 vs. 3.7 ± 0.45 mmol/L in MR and WM; $P = 0.05$), cholesterol (2.8 vs. 3.4 ± 0.15 mmol/L in MR and WM; $P = 0.01$), alkaline phosphatase (312 vs. 446 ± 64 U/L in MR and WM; $P = 0.01$), and paraoxonase (53.7 vs. 64.6 ± 6 U/L in MR and WM; $P = 0.10$), while having lower bilirubin (5.3 vs. 3.0 ± 1.1 μmol/L in MR and WM; $P < 0.01$) than MR calves. The WM calves had lower percentage of circulating lymphocytes (56 vs. 51.2 ± 4.2% in MR and WM; $P = 0.02$) and at 21 and 28 d, higher leukocyte and neutrophil counts ($P < 0.01$). Red cell distribution width ($P = 0.01$) was higher in WM. Results indicate that, despite similar DMI, WM calves achieved greater BW than MR calves. Although WM has a higher protein and fat content, the energy intake was similar. Moreover, results from the metabolic indicators suggested enhanced nutrient bioavailability in WM.

Key Words: calf, milk, metabolism

1177 Metabolomic investigation revealed that microbial feed additives influenced amino acid and carbohydrate metabolism in calves. O. O. Adelusi^{*1}, D. P. Casper², R. Goodall⁴, I. M. Ogunade³, and U. Y. Anele¹, ¹North Carolina Agricultural and Technical State University, Greensboro, NC, ²Casper's Calf Ranch, Freeport, IL, ³West Virginia University, Morgantown, WV, ⁴MicroBios Inc., Denver, CO.

Neonatal calf diarrhea costs American farmers approximately \$250 million annually in treatment expenses. This study hypothesized that direct-fed microbials could influence the early-life response of calves by enhancing their nutrition and metabolic efficiency. A total of 86 Holstein bull calves, aged 2 to 5 d, were randomly assigned to 4 treatment groups in a 2 × 2 factorial arrangement, utilizing a randomized complete block design. The calves were fed starter feed containing either *Lactobacillus plantarum* (CLP), a culture mix of *Bifidobacterium animalis* and *Lactobacillus animalis* in milk replacer (BBCM), a combination of CLP and BBCM (CMLP), or a plain milk replacer and plain starter as the control. Blood samples were collected from all calves on d 0 and 54 for untargeted metabolomic analysis of plasma using liquid chromatography-mass spectrophotometry (LC-MS). Data were analyzed using MetaboAnalyst 6.0, HMDB and KEGG database were used to annotate the metabolites, and metabolite set enrichment analysis (MSEA) was used to identify pathways altered by the treatments. Differential metabolites were identified using a volcano plot, with metabolites showing a fold change (FC) = 1.2 or = 0.83 and P -value

= 0.05. In total, 231 metabolites were detected. Volcano plot analysis revealed 24 differentially abundant metabolites, with several ketone bodies and amino acid compounds upregulated in CLP, BBCM, and CMLP compared with the control. Notable metabolites including keto-D-fructose, aldehydo-D-mannose, and 5-methyl-5-hexen-2-one were abundant in CLP, BBCM, and CMLP, respectively, whereas furostanol-26-aldehyde was abundant in CMLP. Additionally, rotundifolone and 2D-5-O-methyl-2,3,5/4,6-pentahydroxycyclohexanone were abundant in BBCM and CMLP, whereas 2-methyl-3-ketovaleric was abundant in CLP and CMLP. Eleven metabolic pathways were significantly enriched by the additives. Specifically, amino acid degradation pathways of valine, leucine, and isoleucine were enriched in CLP and CMLP, whereas carbohydrate metabolism pathways such as pentose/glucuronate inter-conversion were enriched in CLP and BBCM. This study demonstrates that microbial additives altered plasma metabolome of calves through changes in the abundances and/or activities of pathways involved in amino acid and carbohydrate metabolism.

Key Words: calf, metabolomics, direct-fed microbial

1178 The effect of ionophores or oregano extract and saponins-based product supplementation on weaned calves after an *Eimeria bovis* infection challenge. C. Rocha^{*1}, D. M. Moreira¹, R. E. Aguirre¹, R. Contreras¹, M. A. Ballou¹, E. Burroughs², and V. S. Machado¹, ¹Department of Veterinary Science, Texas Tech University, Lubbock, TX, ²Anpario plc, Worksop, Nottinghamshire, United Kingdom.

Our objective was to evaluate the effects of oregano essential oils (EO) and saponins (SAP) compared with ionophores on fecal shedding of weaned calves undergoing an *Eimeria bovis* (EB) infection challenge. A total of 109 56-d-old calves (47 Holsteins and 62 beef-on-dairy [BxD]) housed individually in hutches were randomly allocated into negative control (NC), positive control (PC), ION1 (200 mg/d of lasalocid sodium), ION2 (200 mg/d of monensin), NT1 (3 g/d of product containing 5% of EO, Orego Stim Powder), and NT2 (1.5 g/d of product containing 10% of EO and 10% of *Quillaja saponaria* Mol. [SAP]; Orego Stim Plus Powder 2X). Treatments were administered daily throughout the study, and calves from all treatments but NC were orally challenged with EB (~200,000 oocysts in 10 mL of sterile saline) 14 d after enrollment. Fecal samples were collected at enrollment and 7, 14, 21, 28, 32, 35, 38, 42, 46, and 49 d later. Statistical analysis was performed in SAS using a linear mixed model with repeated measures, with breed and initial BW and their interaction with treatment were offered to the model. The fecal EB count was cubic root transformed to achieve normality of residuals. Treatment influenced EB counts at peak EB shedding, but only at 21 post-challenge. The EB counts (oocysts/g, back-transformed) were greater in PC (2,207), ION1 (1,191), NT1 (1,399), and NT2 (656) in comparison to NC (~0) calves ($P < 0.01$). No differences were observed between NC and ION2 (2.5) calves ($P = 0.84$). In comparison to PC calves, the EB count was lower for ION2 and NT2 counterparts ($P < 0.01$). No differences between PC calves were observed when compared with ION1 ($P = 0.14$) and NT1 ($P = 0.44$). Additionally, no differences between ION1 and NT1 ($P = 0.99$), ION1 and NT2 ($P = 0.39$), and NT1 and NT2 ($P = 0.12$) were observed. Treatment ION2 decreased EB count when compared with NT2 ($P < 0.01$). These findings demonstrate the efficacy of NT1 and NT2 in reducing the EB shedding after challenge was comparable to ION1, but it was not as efficacious as ION2.

Key Words: essential oil, saponin, calf

1179 Effects of starter crude protein levels and methionine supplementation on immunity in postweaning Holstein dairy heifers. M. Mancheno Valarezo^{*1}, T. Fernandez-Wallace¹, M. Cid de la Paz¹, T. Naze¹, G. Larsen¹, E. Schwab², N. Litherland², J. Laporta¹, and L. R. Cangiano¹, ¹Department of Animal and Dairy Sciences, University of Wisconsin-Madison, Madison, WI, ²Vita Plus Corporation, Madison, WI.

This study evaluated the in vitro cell proliferation capacity of bovine peripheral blood mononuclear cells (PBMCs) in Holstein heifers fed different starter concentrates with increasing crude protein (CP) levels, with or without methionine (Met) supplementation from 3 to 84 d of age. Sixty Holstein calves were randomly assigned to one of 6 treatments: CP14 (14% CP without Met, n = 10), CP14-M (14% CP with Met, n = 10), CP16 (16% CP without Met, n = 10), CP16-M (16% CP with Met, n = 10), CP18 (18% CP without Met, n = 10), and CP18-M (18% CP with Met, n = 10). All heifers were fed a common milk replacer (Talon 25/25, VitaPlus Corporation) from birth until weaning on d 49. Blood samples were collected a week after weaning (d 56) and 3 wk postweaning (d 77). The PBMCs were isolated and incubated with Concanavalin A for 5 d to track cell proliferation capacity using the reporter Cell Trace Violet. At the end of the incubation period cells were stained using monoclonal antibodies against $\gamma\delta$ T cell receptor, CD4⁺, and CD8⁺ to track specific subsets of T cell proliferation. Data were analyzed using a generalized linear mixed model including the fixed effect of treatment and the random effect of calf nested within treatment. No differences in T cell proliferation were observed on d 56. On d 77, supplementation of Met in the 14% CP starter influenced $\gamma\delta$ T cell proliferation, as evidenced by higher division, proliferation, replication, and expansion indices (CP14 vs. CP14-M; $P < 0.03$). Additionally, at 16% CP (CP16), supplementation of Met also resulted in increased cell proliferation in the $\gamma\delta$ T cell population based on the proliferation index ($P = 0.02$). At 18% CP, no added benefit from Met supplementation was observed on cell proliferation parameters. No significant treatment differences were observed in the proliferation of CD8⁺ and CD4⁺ T cell subsets. These findings suggest that supplementation of Met in starter diets with lower CP levels (14% and 16%) positively influences cell-mediated immune competence in growing dairy heifers and might provide a framework to reduce excess protein feeding while improving health.

Key Words: heifer development, calf health

1180 Impact of early-life feeding strategies on growth and health in Holstein and crossbred calves. C. Wang^{*1}, H. M. Goetz¹, L. Pisoni¹, A. Keunen², J. H. C. Costa³, M. A. Steele¹, and D. L. Renaud¹, ¹University of Guelph, Guelph, ON, Canada, ²Mapleview Agri, Palmerston, ON, Canada, ³University of Vermont, Burlington, VT.

The objective of this randomized controlled trial was to evaluate the effects of milk feeding regimens on the health and growth of dairy calves. A total of 128 male calves (Angus-Holstein, n = 41; Holstein, n = 87), aged 3–10 d, were enrolled in an 84-d study at a calf-raising facility in southwestern Ontario, Canada. Calves were randomly assigned to one of 3 feeding regimens for the first 21 d: (1) low-fat (LF) MR (26% CP, 17% fat); (2) high-fat (HF) MR (26% CP, 24% fat); and (3) extended colostrum (EXT) MR (26% CP, 24% fat, with colostrum replacer comprising 18% of the MR). All calves were fed 6 L of MR daily for the first 3 wk, then transitioned to LF MR with milk intake increased to 8 L/d from d 21 to 42. Calves were then gradually weaned until fully weaned at d 63. Calf starter and water were provided ad libitum throughout

the entire study. Blood was collected on d 1 to measure IgG and body weight was recorded weekly. Fecal consistency and respiratory scores were assessed twice daily. A linear regression model assessed predictors associated with ADG, whereas a logistic regression model evaluated the odds of respiratory disease (respiratory score of >5). Further, a negative binomial model analyzed days with diarrhea (fecal score of >2). Feeding regimen did not affect ADG; however, Holsteins gained 0.13 kg/d less than crossbreds ($P < 0.01$, 95% CI: -0.22 to -0.03). Calves with >26.0 g/L IgG had lower odds of respiratory disease (OR = 0.27, $P = 0.01$, 95% CI: 0.10 to 0.73) than those with <16.4 g/L IgG on d 1. However, feeding regimen was not associated with respiratory disease. Holstein

calves had a higher incidence of diarrhea than crossbred calves (IRR = 1.75, $P = 0.04$, 95% CI: 1.03 to 2.99). Additionally, an interaction was observed between IgG concentration and feeding regimen ($P < 0.01$). Specifically, in calves with <16.4 g/L IgG, those in the EXT group had a lower incidence of diarrhea compared with those in the LF group (IRR = 0.18, $P = 0.001$, 95% CI: 0.07 to 0.50). Extended colostrum feeding reduced diarrhea in calves with low IgG, but breed also plays a crucial role in growth and disease resistance.

Key Words: growth, disease, feeding strategy

ADSA-USD Competition: Original Research Oral Presentations

1181 Effects of heat stress and plane of nutrition on skin morphology of preweaning dairy calves. K. E. Hardy^{*1}, B. D. Davidson¹, M. C. Guenther¹, C. G. Savegnago², S. Tao², and J. Laporta¹, ¹University of Wisconsin–Madison, Madison, WI, ²University of Georgia, Athens, GA.

Heat stress imposes physiological strain on animals, diverting energy from growth and development toward thermoregulation. When nutrient availability is low, organ function and thermoregulation become insufficient. This study examined the combined effects of nutrition and environment on the hair and skin morphology of preweaning dairy calves. At 2 d of age, Holstein heifers were enrolled into an experiment with a 2 × 2 factorial arrangement with 2 planes of milk replacer nutrition (CN; 4 L/d or HI; 9 L/d; Land O'Lakes Cow's Match; 26% CP, 20% fat) and with or without heat abatement in a subtropical summer (CL; cooled via a positive pressure ventilation tube system (PPVT) or HS; heat stressed without PPVT; airspeeds = 1.5 vs. 0.3 m/s, respectively). Calves were individually housed outdoors under a shade structure (average THI = 78.4) with *ad libitum* access to starter grain. Hair and skin samples were collected from the neck at 62 ± 0.2 d (n = 6/treatment; n = 24). Skin biopsy tissue was fixed in 10% formalin, dehydrated, paraffin embedded, sectioned, and hematoxylin and eosin stained to visualize the glandular tissue. Hair length, diameter, skin stratum corneum (SC) area, dermis thickness, number and area of sweat glands (SWG) and sebaceous glands (SBG), and SWG depth were assessed using ImageJ. Data were analyzed using PROC MIXED in SAS with main effects of environment, nutrition, and their interaction. No differences were captured in hair length or diameter, SC area, or the depth of the deepest SWG. The HS heifers had more superficial SWG (HS vs. CL; 0.39 vs. 0.48 ± 0.02 mm, *P* = 0.04), tended to have a thinner dermis (0.59 vs. 0.64 ± 0.02 mm, *P* = 0.10), had more SBG (16 vs. 12 ± 1 gland, *P* = 0.04), and more SWG (19 vs. 15 ± 1 gland, *P* = 0.02). Additionally, the size of SBG and SWG were altered by the interaction of nutrition and environment. Under CN nutrition, HS heifers tended to have smaller SBG (2,601 vs. 4,250 ± 467 mm², *P* = 0.08) and smaller SWG (7,017 vs. 0.10833 ± 1,410 mm², *P* = 0.03). Preweaning HS heifers exhibit more SBG and SWG and thinner skin. Inadequate nutrition in early life may exacerbate these skin alterations, hindering future thermoregulatory ability.

Key Words: thermoregulatory, hyperthermia

1182 Adrenergic receptor β 2 drives lipolysis and UCP1 expression in subcutaneous adipose tissue of dairy cows. P. Castro^{*1}, B. B. Sparks¹, and C. Strieder-Barboza^{1,2}, ¹Department of Veterinary Sciences, Davis College of Agricultural Sciences and Natural Resources, Texas Tech University, Lubbock, TX, ²School of Veterinary Medicine, Texas Tech University, Amarillo, TX.

Adipose tissue (AT) lipolysis plays a crucial role in supporting negative energy balance in early-lactating dairy cows. Beta-adrenergic receptors 1, 2, and 3 (ADRB) are modulators of AT lipolysis. However, how activation of distinct ADRB contributes to AT lipolysis in dairy cows remains unclear. We investigated how the activation of β 1, β 2, or β 3 adrenergic receptors change glycerol release and gene expression of lipid metabolism markers using an AT ex-vivo model. Perirenal (PRAT) and flank subcutaneous (SAT) AT were collected from 4 Holstein cows at a local abattoir, 500 mg-explants were incubated for 2 h in one of 7 treatments: control, non-stimulated; 10 μ M dobutamine (β 1-agonist); 100 μ M procaterol (β 2-agonist), 10 μ M CL316,243 (β 3-agonist), 1 μ M isoproterenol

(pan- β -agonist), 10 μ M L-748,337 (β 3-antagonist), and β 3-antagonist + pan- β -agonist. Expression of peroxisome proliferator-activated receptor gamma (PPARG), perilipin-1 (PLIN1), hormone-sensitive lipase (LIPE), uncoupling protein-1 (UCP1), proliferator activated receptor gamma coactivator-1 α (PGC-1 α), early B-cell factor-2 (EBF2), and transmembrane protein 26 (TMEM26) were quantified by RT-qPCR. Glycerol concentrations in the medium were quantified by colorimetric assay and normalized to sample weight. Statistical analysis was performed via PROC GLM in SAS. In SAT, treatment with β 2 agonist increased glycerol release over to β 1-agonist (*P* = 0.03), β 3-antagonist (*P* = 0.004), and non-stimulated (*P* = 0.001), whereas no differences were evident in PRAT explants. Notably, stimulation of the β 2 receptor in SAT increased UCP1 expression vs. the β 3-agonist (*P* = 0.04), pan- β -agonist (*P* = 0.02), and the non-stimulated (*P* = 0.02), suggesting a thermogenic effect of β 2 activation. In PRAT, UCP1 was increased by β 3-agonist compared with β 2-agonist (*P* = 0.06), β 3-antagonist (*P* = 0.05), β 3-antagonist + pan- β -agonist (*P* = 0.03), pan- β -agonist (*P* = 0.05), and the non-stimulated (*P* = 0.01). Our results suggest a depot-specific regulation of oxidative phosphorylation by UCP1 by β 2 and β 3 ADRB in SAT and PRAT, respectively, with a marked stimulation of lipolysis dependent on β 2 receptor in SAT.

Key Words: adipose tissue, beta-adrenergic receptor (ADRB), lipolysis

1183 A comparison of in vitro disappearance among alternate forage sources. S. E. McNichols^{*}, N. E. Rehder, and T. L. Felix, Penn State University, University Park, PA.

With shifting weather patterns and increased emphasis on efficiency throughout the entire production cycle, dairy cattle producers need options. In particular, forages outside of the traditional corn silage and alfalfa-based systems have growing interest due to their ability to fit in double, or alternative, cropping systems. This study's objective was to assess the nutrient concentrations and in vitro digestibility of various forage sources, including corn silage (CS), sorghum sudangrass haylage (SSG), oatlage, triticale, forage sorghum, and wheatlage. These forages were chosen for their ability to fit into a variety of rotational cropping systems and changing environments. Forage samples were incubated for 2, 4, 8, 12, 48, 72, and 96 h of in vitro digestion (n = 4; ANKOM Technology method 3, ANKOM DAISYII Incubator, ANKOM Technology, Macedon, NY). Post-incubation, samples were analyzed for dry matter (DM; n = 2; AOAC method 930.15) and neutral detergent fiber (NDF; n = 2; ANKOM Technology method 6, ANKOM200 Fiber Analyzer) to calculate dry matter disappearance (DMD) and neutral detergent fiber disappearance (NDFD). Data were analyzed using the MIXED procedures in SAS, with forage source and hour and their interaction as fixed effects in the model. Forage source, hour, and the interaction of source × hour differed (*P* < 0.05) for both DMD and NDFD. When comparing DMD among forages, CS was fermented the fastest, with nearly 50% DMD within the first 12 h of the in vitro fermentation. All other forage sources did not surpass 50% DMD until after 24 h and close to 48 h. The relative proportion of easily degradable starch in corn silage compared with other forage sources drives these differences. By the 8 h in vitro fermentation, CS also had the greatest fiber disappearance among all the forages at 60.2% NDFD. Across all forages sampled, in vitro fermentation began to plateau by 48 h post-incubation. However, the other forages ranged between 38.4% and 52.3% NDFD by 8 h. Overall, the relative lack of differences among forage sources, other

than corn silage, suggests producers have a variety of feed options to select from for their cattle.

Key Words: dry matter disappearance, forage, in vitro

1184 Effects of balancing dietary 18-carbon fatty acids from high oleic soybeans or a commercially available stearic acid-enriched supplement on milk production of mid-lactation dairy cows. B. A. Seelenbinder*, J. E. Paraless-Girón, A. M. Bales, and A. L. Lock, *Michigan State University, East Lansing, MI.*

We determined the short-term impact of balancing dietary 18-carbon fatty acids (FA) from high oleic soybeans (15.4% DM total FA; 85% oleic acid [C18:1]) or a commercially available stearic acid-enriched fat supplement (97.3% DM total FA; 52% stearic acid [C18:0]) on milk production of dairy cows. Thirty-two Holstein cows (49.7 ± 5.76 kg/d, 120 ± 33 DIM) were used in a crossover design experiment with 2 consecutive 14-d periods, with sampling the last 4 d of each period. Treatments were a diet containing 8% diet DM roasted and ground high oleic soybeans (HOS) or a diet including 2% diet DM of a stearic acid-enriched fat supplement (SAT). Conventional soybean meal, heat-treated soybean meal, and soyhulls were utilized in differing amounts to maintain diet nutrient composition of (% DM) 21% forage NDF, 28% starch, and 17% CP (46% RUP). A palmitic acid (C16:0)-enriched supplement was included in HOS to ensure similar dietary C16:0 content. Diets contained (% total FA) 24 vs. 23% C16:0, 3 vs. 24% C18:0, 37 vs. 13% C18:1, and 73 vs. 70% total 18-carbon FA for HOS and SAT, respectively. The statistical model included the random effect of cow within square and the fixed effects of treatment, period, square, and their interactions. Compared with SAT, HOS decreased DMI (31.2 vs. 32.0 kg/d; $P = 0.03$), increased the yields (kg/d) of milk (50.8 vs. 48.5 ; $P = 0.01$), milk protein (1.58 vs. 1.51 ; $P = 0.02$), milk lactose (2.49 vs. 2.39 ; $P < 0.01$), 3.5% FCM (55.1 vs. 53.0 ; $P = 0.04$), and ECM (54.4 vs. 52.1 ; $P = 0.03$), and tended to increase milk fat (2.05 vs. 1.97 ; $P = 0.07$). Additionally, HOS increased short-term BW gain (0.78 vs. -0.01 kg/d; $P < 0.01$) compared with SAT. Despite balancing diets for total, 16-, and 18-carbon FA in HOS and SAT, supplying 18-carbon FA mainly as oleic acid from high oleic soybeans improved milk and milk component yields compared with supplying stearic acid from a commercially available stearic acid-enriched supplement. This emphasizes the importance of considering individual 18-carbon FA in diet formulations rather than grouping them collectively.

Key Words: oleic acid, stearic acid, milk production

1185 Effect of rumen-protected methionine supplementation on biochemical parameters in mid-lactation primiparous Holstein cows. V. A. de Oliveira*, C. R. Monteiro¹, G. M. de Souza¹, J. P. A. Rezende¹, L. H. Silva¹, T. L. Resende², and M. A. C. Danes¹, ¹*Department of Animal Science, Federal University of Lavras, Lavras, MG, Brazil*, ²*Evonik Industries, Sao Paulo, SP, Brazil.*

High summer temperatures negatively affect dairy cow performance and health by disrupting metabolic processes. Methionine (Met), an essential amino acid, supports protein synthesis and antioxidant defense, whereas blood metabolites serve as indicators of responses to heat stress. This study evaluated the effect of rumen-protected Met (RPM) supplementation on biochemical parameters in lactating dairy cows during the summer. The experiment was conducted in randomized blocks over 9 wk in Brazil. Eighty primiparous Holstein cows (43.7 ± 4.0 kg/d of milk; 182 ± 63 DIM) were paired by milk yield and DIM and

assigned to 2 diets: a control diet or 0.75 g/kg RPM (Mepron, Evonik Industries) in the TMR. Cows were housed in a compost barn with 2 pens. Plasma samples were collected at 12:00 p.m. in wk 3, 6, and 9. The model included treatment, week, and treatment $\times$ week interaction as fixed effects, with block and cow as random effects. Least squares means were compared using Tukey's test ($P \leq 0.05$). Supplementation with RPM did not affect glutamic-oxaloacetic transaminase (111.3 vs. 108.0 U/L, $P = 0.35$), glutamic-pyruvic transaminase (20.2 vs. 21.1 U/L, $P = 0.17$), cholesterol (207.1 vs. 211.9 mg/dL, $P = 0.45$), high-density lipoprotein (95.3 vs. 95.5 mg/dL, $P = 0.89$), albumin (3.4 vs. 3.4 mg/dL, $P = 0.36$), or urea (36.5 vs. 36.0 mg/dL, $P = 0.13$). Plasma glucose was lower in the RPM group (39.3 vs. 43.2 mg/dL, $P < 0.01$). No differences were observed for haptoglobin (20.4 vs. 15.8 mg/dL, $P = 0.24$) or immunoglobulin A (99.1 vs. 106.7 mg/dL, $P = 0.19$). Supplementation of RPM did not affect glutathione peroxidase (0.3 vs. 0.3 U/mL, $P = 0.65$) or superoxide dismutase (44.3 vs. 43.2 U/L, $P = 0.71$), but plasma malondialdehyde (MDA) levels were lower in the RPM group (2.5 vs. 2.8 nmol/mL, $P = 0.01$). The observed blood responses correspond with increased milk production in the RPM group (44.9 vs. 42.9 kg/d, $P < 0.01$). Reduced MDA levels suggest that RPM supplementation may alleviate oxidative damage, while lower glucose levels indicate enhanced energy utilization, contributing to improved milk production.

Key Words: amino acid, biomarker, heat stress

1186 Precision dairy technology adoption in Texas dairy farms. E. Haugen* and S. Paudyal, *Texas A&M University, Department of Animal Science, College Station, TX.*

The objective was to understand the adoption and the perceived usefulness of precision technologies by dairy farmers in Texas. Dairy farmers were delivered printed copies of a pretested survey instrument to assess technology use, on-farm decisions, and producers' planned behavior related to technology adoption on farms. Thirty-three completed surveys were returned and analyzed using descriptive statistics in Microsoft Excel. Most of the respondents used daily milk weights (53%), followed by rumination sensors (33%), activity monitors (26%), cow sorting gates (20%), and automatic feed pushups (13%), whereas 26% of respondents indicated using none of the available technologies. On a scale of 1 to 5 (least useful to very useful), respondents scored the greatest perceived usefulness for estrus detection (mean 4.25; range 1–5), followed by daily milk yield (3.88; 3–5), rumination time (3.71; 3–5), milk components (3.63; 1–5), milk conductivity (3.14; 1–5), BCS (2.85; 1–5), and rumen pH (2.0; 1–4). When choosing a technology, respondents ranked (on a scale of 1 to 5; unimportant to very important) the availability of local tech support as the most important factor (score 4.16; range 3–5), followed by proven performance through research (4.0; 3–5), simplicity of use (3.82; 3–5), benefit to cost ratio (3.66; 2–5), and total cost of investment (3.5; 1–5). Other factors to be very important in the decision stated by respondents include high upkeep costs, technological complexity, and skills of the current labor force. Regarding barriers to investment decisions, 26% ranked lack of access to capital as a primary issue, followed by environmental regulations (12.5%) and political environment (12.5%). Knowing what they know now about technology, 40% would invest the same way, 6.25% would have invested even quicker, and 12.5% regretted making the investment. We conclude a wide variation of producer experience with precision dairy technologies, which presents opportunities for the industry to improve technology adoption and subsequent farmer experiences.

Key Words: dairy farm, technology, adoption

1187 Bacterial spores in a dairy processing environment represent diverse subtypes and vary in concentrations by time and sampling site. W. J. Bollum*, R. L. Weachock, and N. H. Martin, *Cornell University, Ithaca, NY*.

Bacterial spores, which are found in raw milk and have the ability to survive the pasteurization process, are a concern for product spoilage and foodborne illness. Spores are found in food processing environments and may be introduced into products through cross-contamination or insufficient cleaning and sanitation. Here, we aim to enumerate and identify sporeforming bacteria isolated from 15 environmental sites in a dairy processing facility over 3 sampling trials. Mesophilic spore tests were performed on the environmental samples ($n = 45$). Sponges collected from the processing facility were heat treated at 80°C for 12 min, followed by both a most probable number technique to enumerate low level contamination, as well as pour plating on standard methods agar. The mean spore count across the 15 environmental sites ranged from -1.34 to 3.86 log cfu/mL with a standard deviation of 1.48 log cfu/mL, and variation within site across the 3 sampling events ranged from 0.03 to 2.19 log cfu/mL. Isolates representing unique colony morphologies were characterized using *rpoB* sequencing. The 161 isolates comprised 15 genera, 27 species, and 32 *rpoB* allelic types, including 58 isolates that represented new allelic types not previously described. The most common genera were *Bacillus* and *Paenibacillus*, representing 34.6% and 18.8% of the isolates, respectively. Other genera included *Cytobacillus* (15.0%), *Niallia* (8.27%), and others. Of the *Bacillus* isolated from the processing environment, *Bacillus cereus* s.s. was the most commonly isolated. Further, we observed sporeformers with the same *rpoB* allelic types over multiple sampling events from the same sites, suggesting putative persistence in the processing environment. Overall, our results indicate that dairy processing environments may harbor spores of relevance to dairy product quality and safety. Future studies should further examine spores in processing environments and develop strategies to prevent their introduction into dairy products to reduce food waste, extend product shelf-life, and reduce risk to public health.

Key Words: sporeforming bacteria, environmental sampling

1188 Characterization of biomarkers for pregnancy and lipid metabolism in A₂A₂ Jersey cattle peripheral blood mononuclear cells (PBMCs). C. Russell*, S. J. Ikuejamoye-Omotore, A. E. Kerr, and L. M. Mayo, *North Carolina Agricultural & Technical State University, Greensboro, NC*.

Early pregnancy detection is necessary for dairy cattle to reduce days open and start lactation sooner. However, most early pregnancy detection research is performed with Holsteins between d 25–32 after breeding. The objective was to find a reliable pregnancy biomarker before d 25 after breeding and a biomarker of lipid metabolism specific to Jersey cattle. The hypothesis was that selected pregnancy biomarkers could be detected as early as 14 d after breeding in Jersey cattle blood samples. A₂A₂ Jersey heifers ($n = 12$) and cows ($n = 28$) were enrolled into 5-d CIDR-Synch with GnRH and 2 PGF_{2 α} or OvSynch 48 synchronization protocols, respectively. The day of timed artificial insemination (TAI) was considered d 0 and blood samples were collected 14, 18, 22, and 25 d after TAI for PBMCs RNA isolation and plasma progesterone. Pregnancy was confirmed with whole blood pregnancy associated glycoproteins (PAG) on d 25–28 and transrectal ultrasound on d 32–40 after TAI. Statistical analyses were performed by ANOVA using the MIXED and GLIMMIX procedures in SAS Viya. There was no difference in PAG and ultrasound results ($P > 0.05$) with 33% ($n = 24$), 25% ($n = 55$), 56% ($n = 25$), and 100% ($n = 2$) pregnant for 106 services of heifers, first, second, and third parity, respectively. Preliminary results indicate that ISG15 and FABP3 can be used to detect non-pregnancy and changes in lipid metabolism in Jersey cattle. Further analysis is needed to determine the relationship between lipid metabolism biomarkers and early pregnancy biomarkers in Jersey cattle.

Key Words: early pregnancy detection, A₂A₂ Jersey cattle, biomarker

ADSA-USD Competition: Dairy Production Oral Presentations

1189 The role of vermiculture (earthworms) in the sustainability of dairy farms. R. Lyons*, E. Lindner, and A. De Vries, *University of Florida, Gainesville, FL*.

In a rapidly growing world, agricultural producers must evolve and adapt to feed the global population while considering the operation's efficiency and sustainability. The dairy industry is under intense pressure to reduce the environmental footprint of production, with carbon and manure management at the forefront of these concerns. In response, the dairy industry has committed to neutralizing greenhouse gas emissions by 2050, while also promoting water recycling and reducing runoff. Liquid storage of manure is a leading contributor of greenhouse gas emissions from the industry, leading to air and water pollution. Vermiculture, which is the use of earthworms to break down manure, poses an interesting opportunity for dairy producers to better capture and cycle nutrients through their operation. Vermifiltration systems utilize nature's principles of gravity and microbial metabolism to aerobically treat wastewater, while capitalizing on the value of manure, called vermicompost, and carbon credits in current markets. Although not yet widely adopted, several pilot studies have shown a significant potential of vermifiltration to reduce the concentration of organic matter, nutrients, and solids in dairy wastewater. These research trials have indicated significant reductions in ammonia, methane, and nitrous oxide emissions ranging from 72% to 100% from wastewater and manure management systems. Aside from this system's efficiency in treating wastewater, it also provides advantages in low cost and labor inputs when compared with other alternatives. The implementation of vermiculture systems in dairy operations provides a viable solution to improve operation sustainability and provide another source of income for producers.

Key Words: sustainability, vermiculture, manure management

1190 Embryo loss in the dairy industry. M. Griow* and G. Carpenter, *Iowa State University, Ames, IA*.

Embryo loss in the dairy industry can be significantly detrimental to reproductive efficiency and farm profitability. Embryo loss primarily occurs within the early stages of gestation. A meta-analysis was conducted on 19,723 pregnancy diagnosis records in dairy cows. It was found that pregnancy losses occur in approximately 27% of early embryonic stages (19–32 d) and 13% in the late embryonic stages (30–45 d). Initial pregnancy diagnosis occurs during the late embryonic/early fetal stage of gestation. This means up to 27% of pregnancies could be lost before the initial pregnancy diagnosis is confirmed. Reproductive efficiency is pivotal in determining a dairy herd's profitability, as it directly affects optimal milk production as well as culling decisions. High-producing dairy cattle are especially susceptible to embryo loss due to the negative energy balance they experience and the hormonal distributions that may affect embryo viability. The factors relating to pregnancy losses are put into 2 categories: infectious and noninfectious. Some key contributors to embryo loss include physiological imbalances, nutritional deficiencies, heat stress, genetic predisposition, and infectious diseases. The consequences of embryo loss go beyond reproduction, having an economic impact, reduced herd reproductive efficiency, and increased veterinary and management costs. However, there are many strategic management and preventative practices that can help mitigate the risk of embryo loss. Those key interventions include optimizing nutrition, improving heat stress management, enhancing reproductive management, implementing enhanced control over infectious diseases,

and improving genetic selection. Recognizing that embryo loss is important for maintaining reproductive productivity is key to guaranteeing long-term sustainability within the dairy industry.

Key Words: embryo loss, reproductive efficiency, high-producing dairy cattle

1191 Use of precision dairy technology to evaluate body condition scores. W. Robey* and D. Winston, *Virginia Tech, Blacksburg, VA*.

Body condition score (BCS) is a widely recognized tool in the dairy industry, aiding in herd health management by assessing body fat reserves in dairy cows. Different scoring systems exist globally, but all follow the principle that lower scores indicate thinner cows, whereas higher scores signify heavier animals. Body condition scoring is particularly critical around calving, as cows naturally lose condition due to hormonal changes and lactation demand. Maintaining an optimal BCS at calving (3.0–3.5 on a 5-point scale) is essential for productivity, reproductive performance, and overall health. Deviations from this range can lead to reduced milk yield, metabolic disorders, and compromised feed intake. Despite its importance, traditional BCS methods are labor-intensive, subjective, and impractical for large-scale dairy operations. Emerging automated BCS technologies utilizing 3D cameras offer a promising solution. These systems can capture multiple scores per day, improving monitoring precision and eliminating human subjectivity. Studies have demonstrated that multi-camera setups enhance accuracy by evaluating numerous body regions, surpassing the limitations of single-camera approaches. Automated systems have also proven more sensitive in detecting BCS changes, identifying variations significantly earlier than visual assessments. One study found that whereas manual scoring required 44 d to detect changes, refined automated methods could do so in 12 d. Such advancements enable earlier intervention for metabolic diseases and optimize transition cow management. Although automated BCS systems significantly enhance accuracy and efficiency, challenges remain, including calibration for extreme BCS values and the need for broader validation across different breeds. However, the potential benefits—such as improved animal welfare, reduced labor demands, and enhanced dairy management—underscore the value of these technologies. As refinement continues, integrating automated BCS scoring into dairy operations will facilitate more precise, real-time decision-making, ultimately improving herd health and productivity.

Key Words: body condition score

1192 Individual versus pair housing in dairy calves. A. C. Hebert* and C. C. Williams, *Louisiana State University, Baton Rouge, LA*.

Management of newborn dairy calves is critical to their growth and survival. Although much emphasis is placed on colostrum in the first 24 h of life, other management factors are also important. The type of housing can have an impact on health and productivity of the preweaning calf. Individual housing is most often used on US dairy farms, with approximately 70% of producers choosing this type of housing. However, interest in pair housing calves is on the rise. Some important things to consider are how each of these methods affects both the producer and the calf. This includes effectiveness, simplicity, health, socialization, and freedom from both fear and stress. While housing methods do not

have negative effects on the amount of human interaction received and the health of the cattle during the preweaning period, it can negatively affect the postweaning health and social needs of the animals. Studies have shown that individually housed calves were more prone to health issues during the postweaning phase when introduced to a group. These same animals also were more stressed in comparison to the pair-housed calves, as they had received no social interaction with herd mates. A concern for pair housing is the cross suckling that usually occurs. While it is believed that these animals are more prone to health issues, research has shown that there is no significant effect on calf health, especially postweaning. Recent studies have shown trends for increased preweaning growth rate and starter intake in pair-housed calves. Additionally, calves pair-housed preweaning spent more time lying down during the postweaning stage. While some studies have shown improved health and well-being in pair-housed calves, other studies have reported no significant differences in the 2 types of housing. With good management, both housing methods can be effective in producing healthy calves. While paired housing may be more favorable to calf welfare and can be seen as the future, the decision of which housing method to use is ultimately up to the producer.

Key Words: dairy calf, individual housing, pair housing

1193 Using precision technologies to monitor calf health. H. L. Mansfield* and D. R. Olver, *Pennsylvania State University, PA.*

As dairy farms become larger, it becomes more difficult for producers to manage individual animal health and welfare. Precision technologies provide farmers with insightful data that they can use to manage animals more efficiently to minimize morbidity and mortality. Though originally used for older cows, these technology devices have recently been applied to monitoring calf health to improve their welfare, specifically by timely detecting diseases such as pneumonia and diarrhea. Two of the most validated calf technologies are Automatic Milk Feeding Systems (AMFS) and Triaxial Dimension Accelerometers. The AMFS help to increase efficiency by reducing labor needs, increasing milk intake and calf performance, and lessening postweaning stress through automatic gradual weaning. Furthermore, research shows that AMFS measurements of daily milk consumption and drinking speed can be used to predict diseases and death in calves. Accelerometers measure lying and activity time in calves and can therefore detect disease before calves are symptomatic. A variety of other emerging calf technologies are currently being studied and are still in the validation process for commercial use. In addition to validation challenges, integration of data when using multiple technologies can be difficult. In turn, incorrect or incomplete interpretation of the data may translate to poor management decisions and an inefficient use of the technology. As much of this precision equipment requires a high initial investment, it is essential that producers purchase from reputable, validated brands while also knowing how to use the data to implement best management practices. These challenges show the need for further testing of these innovative calf technologies, which should then be integrated to maximize managerial and production efficiencies.

Key Words: precision technology, calf health

1194 Evaluating infrared thermal imaging as a real-time diagnostic tool for mastitis detection. M. Coffman*, S. Dupont, and J. Bohlen, *University of Georgia, Athens, GA.*

Mastitis alone accounts for approximately 60% to 70% of antibiotic use on dairy operations and costs farmers over \$400 per case as the result

of reduced production, antibiotic cost, and dumped milk. Thus, quick identification and treatment, if necessary, of mastitic cows is essential. However, studies report that more than 60% of cases of mastitis are subclinical and subsequently go undetected, costing the US dairy industry over \$1 billion annually. The most common method to detect subclinical cases is via individual, monthly SCC records; however, this delay can be costly. Therefore, development of alternate strategies to identify these subclinical cows quickly and accurately is needed. Infrared (IR) thermal imaging coupled with artificial intelligence (AI) may serve as a non-invasive method for efficient, real-time temperature monitoring for mastitis identification. Widespread use of IR thermal imaging is already present in the human medical field leading to advancements such as the early detection of certain cancers. Although studies currently vary in efficiency and accuracy of IR imaging for mastitis detection, the interest in implementing IR for a variety of dairy production needs is growing. Recent research using IR technology to detect subclinical mastitis found that an increase in udder temperature, specifically the left and right forequarters, was highly correlated with SCC. It is realized that ambient temperature may play a part in changing body temperature; however, the usefulness may be in imaging changes over time as well as between images within udder. There is concern that all bacteria do not yield similar results, as species like *Escherichia coli* cause an increase in udder surface temperature by 1°C to 1.5°C, whereas some mastitis-causing species like *Staphylococcus chromogenes* result in no fevers nor image changes despite having the same production consequences. Inconsistency in study conclusions highlights current limitations with variables such as anatomical region, environment, and pathogen impacting results. While these warrant further investigation, there is promise that further work and validation of IR imaging could prove viable for reducing the financial implications of mastitic infections and improve overall animal health.

Key Words: thermal imaging, mastitis, disease detection

1195 Estrus and ovulatory abnormalities due to increased hepatic metabolism of steroid hormones in lactating dairy cows. M. Penner* and S. Paudyal, *Texas A&M University, Department of Animal Science, College Station, TX.*

Recent studies have indicated that lactating dairy cattle have reduced estrus expression, standing heat, increased rates of multiple or double ovulations, longer time from luteolysis to ovulation, larger corpus luteum, and smaller progesterone peaks when compared with beef cattle. High-producing dairy cows consume more feed in response to high metabolic demands which directly shifts the hepatic portal veins and hepatic artery to circulate a higher proportion of liver blood flow (LBF) to the gastrointestinal tract and then back to the liver (increasing from 757 L/h in nonlactating cows to 1,183 L/h in lactating cows). The high blood flow to the liver causes an increase in the metabolism of nutrients including important steroidal hormones that are important for ovulation, conception, and maintenance of pregnancies. The increase in reproductive anomalies in dairy cattle can be traced back to the increased metabolism of steroidal hormones in particular progesterone (P4) and estradiol-17B (E2) due to higher quantities of LBF and hepatic metabolization. A group of nonlactating and lactating dairy cattle were compared when measuring the systemic circulation levels of P4 and E2. Results have shown that progesterone levels in lactating cows were an average of 2.43 (ng/mL), whereas in nonlactating cows, the measured average was 3.53 (ng/mL). A similar correlation was reported when comparing E2 levels in both groups of dairy cattle, 351.19 (pg/mL) for nonlactating cows vs. 265.44 (pg/mL) for lactating cows. The evidence shows that this relation of LBF may be the underlying cause of the

decreased circulating steroid hormone levels. Low levels of E2 lead to poor estrus expression, which affects increased monetary loss in labor, semen, and overall longer time open. Low levels of estradiol are also directly correlated with double ovulations, with the potential for twin pregnancy, which carries a negative economic output costing between \$59 and \$161. We conclude that the increased metabolism of naturally occurring hormones in lactating dairy cattle affects the reproductive performance of dairy cows and subsequently overall economics of the dairy farm.

Key Words: hepatic metabolism, estrus, reproduction

1196 Beyond the barn: The promise of pasture-based dairy farming. A. Flynn* and E. Eckelkamp, *University of Tennessee, Knoxville, TN*.

Pasture-based dairy operations have the potential to be more appealing to consumers and more comfortable for cows compared with confinement dairy operations. Pasture-based operations may face challenges with nutrition and production efficiency. Additionally, heat stress is a common issue especially in warmer months. Even though pasture-based systems have issues, there are some potential solutions. Supplemented TMR and different grazing methods have potential to assist with the nutrition needed for dairy cows to perform. A study of cows in pasture,

pasture with TMR, and confinement found that cows offered TMR at night and pasture during the day had similar DMI to cows in a confinement system (25.2 vs. 26.7 ± 0.05 kg DM/cow/d, respectively) with both outperforming pasture alone (21.6 ± 0.05 kg DM/cow/d). Milk production responded similarly, with pasture having the lowest production compared with pasture with TMR and confinement (28.5 vs. 32.0 vs. 38.1 ± 1.2 kg/cow/d, respectively). Alternative forage species may also improve pasture performance. A high sugar variety of ryegrass maintained higher milk production compared with conventional ryegrass (15.3 vs. 12.6 ± 0.9 kg/cow/d, respectively). Tools such as misters and shades can be put to use to add heat abatement in pasture settings. In a pasture setting, cows provided shade on pasture produced 2.45 kg/cow/d more than cows without shading available. Other resources state that shades in a grazing system reduced time at the water trough (6.4 times less) and improved lying time (1.7 times more) while increasing grazing time (1.5 times more) compared with cows without shade. When misters were used in conjunction with shade, milk yield increased from 28.1 to 30.5 kg on test day and increased fertility rate from 17% to 25%. Overall, pasture-based dairy operations have challenges. Even so, there are aspects of this management strategy that have potential to support the dairy industry.

Key Words: heat stress, grazing, forage

ADSA-USD Competition: Dairy Foods Oral Presentations

1197 Compositional and technological traits of raw milk harvested with robotic versus conventional milking systems. D. Jimenez Matos* and S. Paudyal, *Department of Animal Science, Texas A&M University, College Station, TX.*

An increasing number of dairy farms have adopted robotic and sensor technologies, including automated milking systems (AMS). These systems automatically milk cows without a need for human involvement, using data and AI to optimize milk harvest. Literature related to milk composition due to differences in milking systems is scarce. The objective is to evaluate compositional and technological traits of raw milk harvested using AMS versus conventional milking systems (CMS) based on the available literature. Different studies indicate that cows milked with AMS produced milk with lower fat content compared with those milked conventionally. While the milking system itself does not significantly affect the gross composition of milk, variations in milk composition can occur due to extremely short or long milking intervals and effective milk letdown independent of the milking system used. From a cheesemaking perspective, AMS milk may offer advantages over CMS milk due to the size and integrity of milk fat globules. Several studies have shown that AMS increases milking frequency, resulting in a 5% to 10% increase in milk yield compared with the fixed-frequency regimen of CMS. This effect is more pronounced in multiparous cows than in heifers. Although most studies initially reported an increase in somatic cell count (SCC) and a deterioration in udder health following the introduction of AMS, some authors observed a decrease in SCC content after allowing an adaptation period. Additionally, the introduction of AMS has been associated with a slight increase in the freezing point of milk, attributed to residual water from frequent cleaning and rinsing of the system. Current literature does not provide a clear consensus on the effects of milking systems on milk composition. Some studies have reported lower free fatty acid levels in AMS milk associated with increased milking frequency, which can negatively impact the technological properties and sensory characteristics of milk, such as whipping ability and taste. By ensuring that technological advancements in dairy farms do not compromise the nutrient profile of milk products, sustainable adoption of technologies can be achieved.

Key Words: robotic milking, composition, raw milk

1198 Exploring the impacts of contamination by per- and polyfluoroalkyl substances in dairy products. T. Tauke*, I. Nielsen, and A. Carpenter, *Iowa State University, Ames, IA.*

Per- and polyfluoroalkyl substances (PFAS), commonly known as “forever chemicals,” are a growing concern in the dairy industry due to their persistence, bio-accumulative properties, and potential health risks. These PFAS are found in industrial applications, firefighting foams, and consumer products. Their environmental stability has led to widespread soil, water, and livestock feed contamination. Dairy cattle exposed to PFAS through these sources may accumulate and excrete these chemicals into their milk, raising concerns about dairy product food safety. Studies indicate that perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), 2 of the most studied PFAS compounds, can transfer from contaminated feed and water into cow milk. Physiologically based pharmacokinetic modeling suggests that a notable portion of ingested PFOS is excreted through milk, with variability depending on exposure duration and concentration. Research on cattle has confirmed PFAS bioaccumulation, with long-chain compounds persisting in tissues due

to limited renal excretion. Findings indicate that PFAS preferentially binds to milk proteins and fats, potentially altering their distribution in whole, skim, and processed dairy products. While regulatory limits for PFAS in milk are still being developed, the US Environmental Protection Agency’s health advisory has discussed some parameters. The long-term health effects of low-dose PFAS consumption in dairy remain variable, emphasizing the ongoing research needed to mitigate PFAS contamination. It will be essential to strengthen regulatory oversight and implement proactive farm management practices to ensure the safety and sustainability of dairy products in the face of PFAS contamination.

Key Words: per- and polyfluoroalkyl substances (PFAS), contamination, dairy product

1199 Milk: It’s not just for strong bones. A. Roheim* and C. C. Williams, *Louisiana State University, Baton Rouge, LA.*

Milk consumption has long been recommended as a natural source of calcium for bone health in people of all ages. In older individuals, dairy milk is recommended to maintain bone strength and to prevent osteoporosis because it is an excellent source of both calcium and vitamin D. While these benefits of milk have been known for years, recent research has revealed other positive health effects of milk. The objective of this presentation is to focus on brain health in older adults as another possible health benefit of consuming milk. Research conducted at the University of Kansas Medical Center found that milk consumption in adults ages 60 and above may increase brain glutathione (GSH) levels. Glutathione is an important antioxidant that helps reduce oxidative stress, a significant factor in aging and neurodegeneration. The study included adults ages 60 to 89 in which participants were assigned to either a control group, maintaining their usual milk intake, or an intervention group, consuming 3 cups of dairy milk daily for 12 wk. Magnetic resonance spectroscopy (MRS) was used to quantify GSH levels in specific brain regions before and after the intervention. Participants in the intervention group exhibited a statistically significant increase in brain GSH levels relative to the control group. Drinking dairy milk may be linked to increases in the brain’s GSH levels because dairy milk provides cysteine, riboflavin, and calcium, key precursors for GSH synthesis, and potentially contributes to enhanced antioxidant production. The results indicate that higher levels of dairy milk consumption protect cells from oxidative damage in the aging brain. Higher dairy milk consumption may also support and reduce oxidative stress and promote cognitive resilience. While the study contributes to the growing evidence that nutrition plays a significant role in brain health, further research is needed to better understand the long-term effects and benefits of milk consumption in older adults.

Key Words: milk, brain health, aging

1200 Dairy products: An asset to athlete performance. S. M. Kennedy* and D. R. Olver, *Pennsylvania State University, PA.*

Dairy products of all kinds are currently marketed as nutrient-rich by the industry. Labels frequently highlight a high protein content along with a great source of calcium and vitamin D. Although these things are true, these limited details fail to represent the true advantage that dairy offers to high-performing athletes. The purpose of this review is to analyze the components in milk that contribute to athlete performance and recovery. There are multiple beneficial components of milk such as hydration elements, energy content, and essential nutrients that can all

be used for the benefit of athletes. The most abundant mineral in milk, potassium, plays an important role in hydration. According to the 10th edition of the Recommended Dietary Allowances, potassium acts as a cation maintaining fluid balance and plays an important role in nerve impulses. The adequate intake for potassium is 3,400 mg for males and 2,600 mg for females. One cup of whole milk provides over 10% of a male's daily potassium requirement, contributing to the essential electrolyte balance. Additionally, the recovery aspect of an athlete's routine is important and can contribute to game-time performance. Milk has been found to be beneficial to the immediate and extended recovery of muscles. A study by Newcastle University analyzed the effect of milk as a post-workout drink and found that it can help reduce the loss of muscle function in explosive movements. This may be due to the composition of proteins in milk providing a fast and slow source of amino acids. In addition to protein assisting in muscle recovery after a workout, macronutrients also help to restore glycogen. There is an advantage to combining carbohydrates with protein to speed up glycogen replenishment. The stored energy in glycogen is often used during endurance workouts but must be replenished by the following workout or game to perform explosively. In a more long-term recovery context, milk can contribute to better sleep. Milk contains tryptophan, magnesium, and zinc, which are all active in melatonin production. The effect of melatonin is prominent in restful sleep. With better sleep, athletes are able to rest and recover, improving next day performance. The use of dairy products for high-performance athletes is often not the focus of marketing campaigns. However, research shows that milk is beneficial to athletes, allowing room for growth in this sector of dairy sales and promotion.

Key Words: recovery, hydration, athletic performance

1201 Type A H5N1 virus reigniting the conversation around the concern of unpasteurized milk. K. Glassman*, S. Dupont, and J. Bohlen, *University of Georgia, Athens, GA.*

Unpasteurized milk, commonly known as raw milk, is associated with significant health risks due to the potential presence of harmful pathogens. Unlike pasteurized milk, which undergoes a heating process to eliminate disease-causing microorganisms, raw milk can harbor bacteria such as *Salmonella*, *Escherichia coli*, *Listeria*, and *Campylobacter*, all of which can lead to severe foodborne illnesses. While the Food and Drug Administration (FDA) supports measures to protect product safety, such as pasteurization, it does not regulate intrastate sale or distribution of raw milk. Despite studies and warnings from public health officials, sales or distribution of raw milk is permitted in more than half of states and raw milk consumption has risen by 65% in recent years; with consumers citing health, taste, and production practice preferences. This trend continues even as the Centers for Disease Control and Prevention (CDC), FDA, and other public health authorities warn against consuming raw milk. The novel identification in March of 2024 of highly pathogenic avian influenza A (HPAI H5N1) in dairy cattle raises new questions with regard to the safety of raw milk products. Currently, documented H5N1 infections cause high morbidity and mortality in poultry, low morbidity and mortality in dairy cattle, and a conjunctivitis-like infection in humans. While much attention has been given to its transmission

via direct contact with infected poultry, dairy cattle, or contaminated surfaces, the safety of food products, particularly unpasteurized milk, raises questions. In a National Institutes of Health (NIH)-funded study, researchers found that H5N1 could survive in raw dairy milk for at least 5 wk when refrigerated, but under temperatures that mimicked those used in milk pasteurization, heat was able to inactivate the H5N1 virus. This study also found that mice who consumed infected raw milk exhibited symptoms of illness, suggesting that consumption of H5N1 infected raw milk could transmit it to consumers. As H5N1 has become a part of the US dairy industry landscape, now affecting more than 15 states, it is crucial that national and state organizations employ greater vigilance over raw milk consumption and ensure effective preventative measures to protect public health as well as enhance informational resources to educate consumers about the potential risks of raw milk consumption.

Key Words: H5N1, raw milk, safety

1202 Diversity and carbohydrate utilization of dairy-associated *Leuconostoc* spp. L. Rael*, R. Weachock, C. Loomis, and N. H. Martin, *Cornell University, Ithaca, NY.*

Chocolate milk is an important dairy beverage, but previous studies have indicated that bacterial growth in chocolate milk occurs faster than in unflavored milk, resulting in sensory defects. One such defect that has been observed is the development of a slimy phenotype caused by *Leuconostoc* spp. This phenotype can pose a significant threat to the shelf life and consumer acceptance of chocolate milk. Here, we aimed to characterize 113 *Leuconostoc* spp. previously isolated from dairy relevant samples to (i) determine the diversity of dairy relevant *Leuconostoc*, (ii) assess carbohydrate utilization for unique *Leuconostoc* sequence types, and (iii) evaluate the ability of *Leuconostoc* to form defects in different milk products. To that end, 113 *Leuconostoc* isolates in our microbial collection were subjected to 16S rDNA PCR and sequencing. Resulting sequences were characterized into unique 16S rDNA sequence types (16S ST), and representatives were chosen from each 16S ST to be evaluated for carbohydrate utilization characterization using API CH 50 strips. Overall, we identified 8 distinct 16S ST with representing 1 to 48 isolates per ST. From these 8 16S ST, we selected 25 *Leuconostoc* isolates to conduct API CH 50. Our results showed great diversity within *Leuconostoc* spp., with inconsistent utilization across the isolates for all but 5 of the carbohydrates tested, including glucose, fructose, and mannose. Conversely, certain carbohydrates, including inulin, amidon, and D-tagatose, were found to be utilized rarely. Within the most common 16S ST, ST 6, there were 9 unique carbohydrate utilization patterns, indicating that the 16S rRNA gene has insufficient resolution to discriminate between isolates exhibiting varying phenotypes. Overall, our results indicate that *Leuconostoc* is a diverse organism, exhibiting varying capabilities to utilize carbohydrates of dairy relevance which will ultimately impact quality deterioration in a variety of dairy products. Future studies should conduct whole-genome sequencing and comparative genomics to determine genotypic drivers for these phenotypic characteristics.

Key Words: *Leuconostoc*, chocolate milk, carbohydrate utilization

Animal Behavior and Well-Being 1

1203 Exploring indicators of positive affective states in dairy cattle. A. Scott^{*1}, G. Dallago¹, H. W. Neave³, J. Ahloy-Dallaire², J. H. C. Costa⁴, K. Plaizier¹, and M. King¹, ¹University of Manitoba, Winnipeg, MB, Canada, ²Université Laval, Québec, QC, Canada, ³Purdue University, West Lafayette, IN, ⁴University of Vermont, Burlington, VT.

While research has mainly focused on negative affective states, it is as important to determine if animals are experiencing positive affect. The objective of this study was to validate behavioral and physiological measures of positive affective states in adult dairy cattle. Using a crossover design replicated Latin square controlling for day and cow effects, 13 dry cows and 5 heifers were each subjected to 3 treatments: positive (POS; large pen, deep straw bedding, brush access, fence-line companion heifer), neutral (NEU; mid-sized pen, minimal bedding, visual of companion heifer), and negative (NEG; small pen, no bedding, no visual contact with other animals, pile of inaccessible pellets). Animals were moved from their group-housed home pens to individual treatment pens, where treatments were applied in random order, with one treatment applied each day for 45 min and 1 washout day between treatments. During each treatment period, we collected heart rate (HR), vocalizations, and continuous video data. Animals were then moved to a test pen where they completed a novel object test (pool toy, hamper, airplane) with continuous video recording. The outcomes of the behavior tests were scored by a single trained observer who was blind to treatments. The data were analyzed using ANOVA, binomial GLM, and survival analysis according to the outcome variable. The HR did not differ by treatment in the initial 15 min. Over the following 30 min (from min 15 to 45), POS had the highest HR ($P = 0.002$), whereas NEU and NEG did not differ until the final 15 min when NEU was the lowest ($P = 0.007$). Vocalization frequency did not differ ($P = 0.48$) by treatment. During the novel object test, approach laterality did not differ, but POS were 8.1 and 13.0 times less likely to approach the novel object during the allotted time than NEU ($P = 0.06$) and NEG ($P = 0.02$), respectively. Our results highlight the validity of heart rate to measure arousal, and that novel object tests may reflect positive affect when evaluating latency to approach. Ongoing analyses include saliva analytes, electroencephalogram recordings, and more detailed behavior data, which may reveal other measures of positive affect.

Key Words: positive affect, novel object

1204 Characterizing individual drinking behavior and productivity of lactating dairy cows under heat stress conditions. M. Bacon^{*1}, T. Da Silva², J. H. C. Costa², and C. Daigle¹, ¹Texas A&M University, College Station, TX, ²University of Vermont, Burlington, VT.

Climate change is a major concern for the dairy industry, as rising global temperatures leave more cows vulnerable to heat stress. Heat stress reduces milk production and compromises animal welfare. Thus, there is a growing need for understanding individual heat stress tolerance in dairy cattle. The objectives of this study were to assess individual differences in the drinking behavior and productivity of dairy cows during time periods with differing levels of risk for heat stress. Data were collected from 22 lactation dairy cows at the T&K Dairy in Snyder, Texas, housed in a freestall barn equipped with HOBO U23 external temperature loggers (Bourne, MA). The temperature-humidity index (THI) was calculated from the daily minimum relative humidity and maximum air temperature. Cows were milked in a free-flow Lely A4

Robotic Milking System (Maassluis, the Netherlands) and fitted with a bolus (SmaXtec, Graz, Austria) for characterizing drinking behavior. Drinking behavior and robotic milking parameters were collected during two 14-d periods. The THI ranged from 54.6 to 65.5 (mean = 60.3) during the comfortable period (CO; November to December 2024) and from 71.2 to 81.0 (mean = 77.9) during the heat stress period (HS; September 2024). To assess differences in the daily milk production and drinking behavior of individual cows, linear mixed-effects models were used with the interaction of cow and period, with a random intercept for cow and water intake or daily milk yield as a covariate. The interaction of period and cow affected both daily milk yield and water intake ($P < 0.001$). Several cows had significant increases in water intake during the HS period compared with CO. Likewise, several cows increased production under heat stress while others were less resilient, suggesting that individual cows have unique responses to heat stress. These results highlight drinking behavior as a potential indicator for heat tolerance in dairy cows.

Key Words: dairy cattle welfare, heat tolerance, precision livestock

1205 Dynamics of drinking water consumption in healthy and sick dairy cows. N. Fagundo^{*}, R. Neupane, B. Shrestha, and S. Paudyal, Department of Animal Science, Texas A&M University, College Station, TX.

This abstract is part of the ADSA-Graduate Student Competition: Production (MS Oral Semifinalists).

The objective was to evaluate the effects of diseases and milk production on the water intake of dairy cows. A retrospective case-control study was conducted using data from an organic dairy farm in central Texas, which consisted of 925,302 data points collected from 898 Holstein dairy cows monitored from 2022 to 2024. Total daily water intake was evaluated to identify the effect of health disorders, focusing on lameness, mastitis, metritis, retained placenta, and others (foot rot, abortion, illness, milk fever, diarrhea). Cow-level variables including lactation stage, parity, and disease records were retrieved from Dairy Comp 305, and daily rumination time, and water intake records were collected using SmaXtec sensor boluses placed in the reticulum post-calving on their first lactation. Daily milk yield was retrieved from Afimilk systems installed on the farm. Statistical analysis was conducted using a mixed-effects model controlled for milk yield, temperature-humidity index (THI), and lactation number with the cow as a random effect in the model. Overall, healthy cows consumed 67.5 L/d; however, compared with dry cows (66.40 ± 0.61 L/d), water consumption was highest in early lactation (73.51 ± 0.61 L/d; $P < 0.01$), followed by mid-lactation (70.86 ± 0.61 L/d; $P < 0.01$) and late-lactation cows (69.81 ± 0.61 L/d; $P < 0.01$). Water intake was greater in multiparous cows compared with primiparous cows (132.46 ± 2.04 vs. 75.83 ± 0.19 L/d; $P < 0.01$) and more in high milk-producing cows compared with low-production cows (66.40 ± 0.61 vs. 48.09 ± 0.30 L/d; $P < 0.01$). Statistically significant differences in water intake between healthy and diseased cows were identified. Cows with lameness consumed less water than healthy cows (65.03 ± 0.14 vs. 66.40 ± 0.61 L/d; $P < 0.05$), as did cows under other diseases (58.53 ± 0.81 vs. 66.40 ± 0.61 L/d; $P < 0.01$). We were not able to detect a significant difference in water intake in cows with clinical mastitis compared with healthy cows ($P = 0.2$). In contrast, cows with metritis consumed more water than healthy cows (72.95 ± 0.30 vs. 66.40 ± 0.61 L/d; $P < 0.01$), and as did cows with retained placenta

(68.49 ± 0.61 vs. 66.4 ± 0.77 L/d; $P < 0.05$). We presented evidence of variable water intake in healthy and sick cows with demonstrated cow and environmental impact, presenting an opportunity for use in disease detection and management.

Key Words: drinking water, dairy cattle, disease

1206 Evaluating the relationship between temperament, productivity, and metabolic status in dairy cows. G. Ceja* and C. L. Daigle, *Texas A&M University, College Station, TX.*

Understanding animal temperament enhances management practices, optimizing production efficiency and welfare. The startle response, a universal reflex to sudden stimuli, serves as a measurable behavioral phenotype that can inform management decisions. We evaluated the relationship between startle response, milk production, and metabolic status in dairy cows, hypothesizing that greater startle response would be associated with lower milk yield (MY) and altered metabolic status, measured via fat-to-protein ratio (FPR). Thirteen Holstein cows (parity 2–3; 57–94 DIM) were studied over a 45-d period on a commercial facility. Daily MY (kg/d), rumination time (RUM; min/d), and FPR were recorded using an automated milking system. After cows entered the dry period, a startle test was conducted. Each cow was individually tested in a $20' \times 20'$ enclosed pen containing one umbrella centered on each of the 4 walls ($n = 4$ umbrellas). Behaviors measured included startle magnitude (MAG; total continuous movement duration post-stimulus, s) and activity (total movement in 1 min, s). Data were analyzed using regression analyses (PROC REG) in SAS 9.4, with significance defined as $P \leq 0.05$. Startle magnitude showed a weak but significant relationship with FPR ($P < 0.01$; $R^2 = 0.17$), while activity exhibited a minimal yet significant association with FPR ($P < 0.01$; $R^2 = 0.03$). Milk yield correlated positively with RUM ($P < 0.01$; $R^2 = 0.45$) and activity ($P < 0.01$; $R^2 = 0.45$) but was not significantly associated with MAG ($P = 0.47$; $R^2 = 0.43$) or RUM ($P = 0.21$; $R^2 = 0.02$). These findings suggest startle response may relate to metabolic status but does not strongly correlate to MY. Additional physiological and management factors (e.g., milkings/d) not included in the model can potentially influence these relationships. Future research should further explore the startle response in individual cows as a behavioral phenotype by incorporating additional physiological and management factors to improve predictive accuracy for production traits.

Key Words: fat-to-protein ratio, milk yield, temperament

1207 Association of personality traits and the expression of sickness behavior in dairy calves with neonatal diarrhea. A. Welk*, H. W. Neave², J. H. C. Costa³, M. C. Cantor⁴, C. B. Winder¹, and D. L. Renaud¹, ¹*Department of Population Medicine, University of Guelph, Guelph, ON, Canada*, ²*Department of Animal Sciences, Purdue University, West Lafayette, IN*, ³*Department of Animal and Veterinary Science, University of Vermont, Burlington, VT*, ⁴*Department of Animal Science, Penn State University, University Park, PA.*

The objective of this study was to assess how personality traits influenced changes in milk feeding and activity behavior in Holstein dairy calves diagnosed with diarrhea. Calves ($n = 30$) housed in groups of 15 were offered up to 15 L/d of milk replacer from an automated feeder, which recorded milk intake and drinking speed. To measure activity behaviors (steps, lying time, and lying bouts), calves were fitted with accelerometers from birth. Calves were fecal scored daily for 21 d. Diarrhea was diagnosed when calves had loose feces for ≥ 2 d or watery feces for ≥ 1 d. Cases were further classified as severe (≥ 4 d with diarrhea) or mild

(<4 d with diarrhea). At 24 d of age, after recovery from diarrhea, calves underwent personality tests over 4 consecutive d. The tests, performed in the sequence of novel environment, novel object, startle stimulus, and social isolation, assessed their behavioral responses. Behavioral responses were analyzed using principal component analysis, identifying 4 traits: shy, fearful, active/vocal, and explorative, which together accounted for 76% of observed variance. Mixed linear regression models assessed the association between personality traits and relative changes in milk feeding and activity behaviors on d 0 of diarrhea, with the baseline set at 0% on d -3 relative to diarrhea diagnosis. In each model, fixed effects included birth weight, diarrhea age, and diarrhea severity, with group pen as a random effect. Shy calves had negative deviations in milk intake ($\beta = -8.4\%$, $P = 0.01$) and increased lying bouts ($\beta = 9.5\%$, $P = 0.02$) on d 0 compared with bold calves. Fearful calves also had negative deviations in milk intake ($\beta = -8.3\%$, $P = 0.01$) compared with non-fearful calves. Active/vocal calves tended to have negative deviations in steps on d 0 ($\beta = -10.8\%$, $P = 0.06$). Relative change in drinking speed and lying time were not associated with the identified personality traits. These results suggest that personality may influence the expression of sickness behaviors during a diarrhea bout, particularly changes in milk feeding and activity behavior for shy and fearful dairy calves.

Key Words: precision technology, coping style, scours

1208 Effect of training dairy heifers to an automated milking system before parturition on their adaptation and performance. J. E. Brasier*, D. B. Haley², R. Bergeron¹, and T. J. DeVries¹, ¹*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada*, ²*Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

The study objective was to determine whether prepartum training of pregnant heifers to an automated milking system (AMS), with concentrate and mechanics exposure (robotic arm and noises), affects their adaptation and performance when milked on an AMS. Thirty pregnant Holstein dairy heifers were enrolled in the study 21 d before their expected calving date and allocated to 1 of 2 treatments: control (CON; no training; $n = 16$) and training (TRN; exposure to the AMS environment, concentrate, and mechanical arm and noises; $n = 14$). The TRN heifers were trained on the AMS 3×/d for 4 d starting at 14 ± 5.1 d (mean $\pm$ SD) before expected calving. All heifers calved alone in maternity pens and were then moved at 4 ± 1.28 DIM (mean $\pm$ SD) to the AMS pen to be milked by the AMS. Ease of entry to the AMS (0 = unassisted to 6 = heavy assistance) and milk letdown (0 = good to 3 = poor) were recorded for the first 6 milkings. Milking behavior and yield were recorded for the first 21 d on the AMS. Continuous focal animal behavior sampling was done from video, on d 1, 3, 7, 14, and 21 of milking on the AMS to quantify time spent at the entrance, exit, and at the crossover of the AMS, while time spent in the fetch pen was recorded for all 21 d (min/d). Data were analyzed using mixed-effect, repeated measures linear regression models. The TRN cows had better ease of entry (2.9 vs. 4.3; SE = 0.24; $P < 0.01$) and letdown (0.4 vs. 0.7; SE = 0.1; $P = 0.02$) scores as compared with the CON cows across the first 6 milkings. The TRN cows also had more voluntary visits (5.6 vs. 4.2 #/d; SE = 0.58; $P = 0.04$), fewer fetching events (0.8 vs. 1.0 #/d; SE = 0.05; $P = 0.04$), spent less time in the fetch pen (14.6 vs. 18.7 min/d; SE = 1.64; $P = 0.04$), and had greater milk yield (32.8 vs. 30.8 kg/d; SE = 0.05; $P = 0.05$) compared with CON cows across the 21-d period. For all cows, time spent at the AMS entrance increased over time (P

< 0.01), while time spent at the exit decreased ($P < 0.01$). Overall, the results demonstrate that training heifers to an AMS before calving can improve adaptation and performance when milked on an AMS.

Key Words: training, robotic milking, behavior

1209 Associations of premilking experience with yield and milk ejection. P. Bacigalupo Sanguesa^{*1,2}, P. L. Ruegg¹, S. Manski¹, R. Thomson¹, B. Norby¹, and R. J. Erskine¹, ¹Michigan State University, East Lansing, MI, ²Michigan State University Extension, East Lansing, MI.

Part of the premilking experience for cows includes walking to the parlor and waiting in the holding pen. The aim of this study was to explore associations between order of entrance to the holding pen, order of milking, and distance walked with yield and measures of milk ejection (ME). Cows ($n = 210$) from 2 groups on 3 commercial farms were enrolled in this study and data were collected for 13 consecutive milkings. All farms milked 3x/d and parlors were equipped with continuous milk flow meters. Cows were uniquely marked on both rump areas to be visually identifiable using time lapse cameras that were placed at the entrance of the holding pens. Recordings were used to determine time of entrance (TE) and parlor recording software was used to obtain time of start of milking (SM), milk yield, and average milk flow between 30 and 60 s for each session. The average milk flow between 30 and 60 s was used to categorize ME as normal or delayed. The TE and SM of each milking of each cow was standardized using percentile ranks of TE and SM. Distance walked was determined using a measuring wheel. The models included nested random effects for cow, group, and farm, and fixed effects to control for the categorical variables of session, parity, stage of lactation, and ME (for yield model). Predictors of interest were distance walked, TE rank, and SM rank. We fit a linear mixed model for yield and a logistic mixed model for ME. The sample had 168 cows. For yield, we found that SM rank and distance walked were associated with yield ($P < 0.05$). Cows with greater SM ranks (later to start their milkings) were associated with higher yields and longer distance walked were associated with a decrease in yield. The TE rank and SM rank were not associated with the occurrence of delayed ME. Distance walked ($P = 0.003$) was associated with a slight increase in odds of delayed ME. Overall, the distance that the cows walk from their housing pens to the holding pen is a relevant variable associated with yield and ME, however additional studies that include more farms should be performed.

Key Words: premilking, yield, milk ejection

1211 Consistency of behavioral traits and their association with feed efficiency traits in lactating Holstein cows. B. M. Nascimento^{*}, H. M. White, F. Peñagaricano, and K. A. Weigel, *University of Wisconsin–Madison, Madison, WI.*

Consistency can be defined as the minimal variation from expected performance over time. High-frequency data from wearable sensors are an interesting source of information to identify consistent dairy cows. The aim of this study was to evaluate the consistency of behavioral traits in lactating Holstein cows and their association with feed efficiency. Data consisted of behavioral and feed efficiency records from 617 cows evaluated at University of Wisconsin–Madison between 2019 and 2023. The behavioral traits analyzed were daily rumination and daily lying time, while feed efficiency traits included average dry matter intake (DMI) and residual feed intake (RFI). Consistency of each behavioral trait was

calculated as the logarithm-transformed variance (logVar) of deviation between observed and expected (average) values. Genetic parameters were estimated using a univariate animal model, including lactation, days in milk, and cohort as fixed effects, and the animal as random effect. Genetic correlations were calculated as Pearson correlation between sires' estimated breeding values. Heritability estimates were 0.13 ± 0.09 for the logVar rumination and 0.16 ± 0.10 for logVar lying. Traits logVar rumination and logVar lying were positively correlated (0.19 ± 0.11), indicating that cows with more consistent rumination also have more consistent lying time. Trait logVar rumination was not correlated with DMI (0.01 ± 0.09) but favorably correlated with RFI (-0.22 ± 0.08). In contrast to most traits, a more negative RFI corresponds to a more feed-efficient animal, therefore the negative correlation is desirable. In addition, logVar lying was moderately correlated with both DMI (0.16 ± 0.08) and RFI (0.12 ± 0.08), indicating that cows with more inconsistent lying time tend to eat more and be less feed efficient. These findings suggest that the consistency of behavioral traits is heritable and could be used as indicators of feed efficiency in dairy cows.

Key Words: rumination time, lying time, residual feed intake

1212 Patterns of behavioral parameters and performance outcomes in lactating cows with automated health alerts and no clinical signs of disease. C. Rial^{*1}, M. L. Stangaferro², M. J. Thomas², and J. O. Giordano¹, ¹Department of Animal Science, Cornell University, Ithaca, NY, ²Dairy Health and Management Services, Lowville, NY.

The objective of this observational study was to evaluate behavioral parameters and herd performance outcomes for cows with automated health alerts (HA) and no clinical health disorders (CHD) diagnosed. Holstein cows ($n = 602$) were fitted with a neck-attached sensor (SenseHub, Merck Animal Health) that monitored rumination time (RT) and activity (ACT) and provided a health index score (HIS). A HA was defined as HIS < 86 arbitrary units, daily RT < 250 min, or both. Milk yield (MY; kg) was monitored 3x/d by milk meters (MM32, DeLaval). A clinical exam was conducted in all cows with HA on the day the alert was generated and every other day until no alert was observed, or until resolution of clinical signs if diagnosed with CHD. Herd exit and first service outcomes were recorded. At 22 DIM, cows were assigned to groups: HA-NCHD ($n = 199$) if had a HA and no CHD, HA-CHD ($n = 168$) if had both HA and a CHD, and NoHA-NCHD ($n = 235$) if had neither a HA nor a CHD diagnosed from 3 to 21 DIM. Binary data were analyzed with logistic regression and continuous data by ANOVA with or without repeated measures. Data are presented as LSM% [95% CI] or LSM $\pm$ SEM. From 2 to 21 DIM, the HA-NCHD group had reduced ($P < 0.05$) RT, ACT, HIS, and MY than the NoHA-NCHD group but greater RT, AT, HIS, and MY than the HA-CHD group. Total MY (kg) to 21 DIM was greatest ($P < 0.01$) for the NoHA-NCHD group (607 ± 2), intermediate for the HA-NCHD group (576 ± 2), and lowest for the HA-CHD group (478 ± 2). From 3 to 21 DIM, the HA-CHD group had more ($P < 0.01$) cows leave the herd (18.1% [11.3–27.7]) than the HA-NCHD (2.5% [0.9–6.4]) and NoHA-NCHD (1.4% [0.4–4.7]) group. Pregnancies per AI were greater ($P = 0.06$) for the NoHA-NCHD (52.9% [45.4–60.4]) than for the HA-CHD (37.6% [27.9–48.2]) and HA-NCHD (46.6% [39.1–54.4]) group. Cows with HA and NCHD may represent a distinct subgroup of cows, with different behavior and performance than cows with HA and CHD and no HA and no CHD diagnosed. Automated behavior monitoring technologies could help identify cows with no evident clinical signs at risk of reduced performance.

Key Words: dairy cow, health monitoring, sensor, rumination, activity

Animal Health 2: Transition Cows

1213 DNA methylation changes and risk loci associated with transition disease. S. E. Jewell*, H. J. Huson, and K. Srikanth, *Cornell University, Ithaca, NY*.

Epigenetic markers, including DNA methylation (DNAm), regulate gene expression in response to environmental stressors and are heritable. They may be involved in the lasting and intergenerational performance effect of disease and could be used to improve genetic selection for disease resistance in dairy cattle. We hypothesized that (1) there are certain DNAm patterns associated with transition disease (TD) risk and (2) there are DNAm changes in response to TD that occur both in the animal that experiences disease and in her future offspring. A cohort of 6 case-control matched primiparous heifer pairs were followed from 1 wk prepartum to 30 DIM and classified on their TD status (TD-H and NoTD-H). A separate cohort of 5 case-control matched dam pairs was identified using electronic farm records and their resulting calves were classified on dam TD status (TD-C and NoTD-C). Whole blood was collected from TD-H and NoTD-H within the week prepartum and at 30 DIM and from TD-C and NoTD-C at birth. The DNAm was assessed via whole-genome bisulfite sequencing at $\sim 7\times$ coverage. Reads were processed and methylation was called with Bismark. Differentially methylated regions (DMR) and genes (DMG) were identified with DMRichR. Comparing TD-H and NoTD-H prepartum, 15,004 DMRs (empirical P -value < 0.05) were identified, 33 of which were further chosen by machine learning (ML) algorithms to classify TD-H and NoTD-H animals. Between prepartum and 30 DIM samples in TD-H, 7,433 DMRs were found and 19 of these DMR were identified by ML algorithms to classify time points within TD-H animals. Last, 12,941 DMRs were found between TD-C and NoTD-C at birth and 19 of these were identified by ML algorithms to classify calves into dam TD groups. The DMG in this study showed significant overlap with DMG and differentially expressed genes associated with transition disease in other published studies including the genes *GRINA* and *RAB28*. The DMR were also enriched in QTL associated with growth and immune functions. Identifying DNAm risk loci and loci that undergo changes in response to TD can improve our understanding of the lasting effects of disease and improve genetic selection of cattle.

Key Words: epigenetics, transition disease, DNA methylation

1215 Adipose tissue neuro-like cell profile changes with ketosis in dairy cows. B. B. Sparks*, H. R. Ford¹, V. S. Machado¹, and C. Strieder-Barboza^{1,2}, ¹*Department of Veterinary Sciences, Texas Tech University, Lubbock, TX*, ²*School of Veterinary Medicine, Texas Tech University, Amarillo, TX*.

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

Activation of adipose tissue (AT) sympathetic nervous system is necessary for lipolysis. Dysregulated lipolysis leads to ketosis in postpartum dairy cows and may involve AT neuro-like cells (NLC). Our objective was to transcriptionally characterize the NLC in AT of cows with sub-clinical ketosis (SCK). Stromal vascular fraction (SVF) was isolated from flank subcutaneous AT of multiparous Holstein cows (mean $\pm$ SD, 3 ± 0.88 parity, 7 ± 1.7 DIM) with SCK ($n = 5$, ≥ 1.4 and ≤ 2.6 mmol/L BHB) or non-ketotic (NK, $n = 5$, ≤ 0.8 mmol/L BHB). Using fluorescent-activated cell sorting, NLC were isolated from the SVF by positive expression of nerve growth factor receptor and negative expression of the immune cell marker CD45 and the mesenchymal

cell marker CD34. The RNA sequencing was performed in SVF (pass 4) and NLC (pass 5) grown in Schwann cell medium. Differentially expressed genes (DEG; $\log_2\text{FoldChange} > |0.2|$, adjusted P -value < 0.05) were identified by DESeq2 package in R (v4.4) and upregulated DEG were analyzed in Enrichr. Independent of SCK, NLC had a similar profile to type IC spiral ganglion neurons (CellMarker 2024), expressing higher *ANKS1B*, *NEGR1*, and *ISLR2* compared with SVF. Functional transcriptomics (SynGO 2024) revealed an activation of the postsynaptic density pathway mediated by *ANKS1B*, *RELN*, and *NEGR1*, thus ratifying NLC neural properties. NK-NLC, expression of lipopolysaccharide binding protein (*LBP*), leukotriene biosynthesis genes *GTT5* and *GTTA1*, neurotransmitter transporter *SLC22A3*, *RELN*, and voltage gated sodium channel, *SCN9A*, were increased in NLC vs. SVF, suggesting a neuroimmune role for NLC in postpartum cows. SCK-NLC had increased lipoprotein receptor 2 (*LRP2*) and *RGS4*, a regulator of lipolysis through catecholamines, and decreased GLUT4 trafficking protein *TRARG1* and glutamate metabotropic receptor 7 (*GRM7*), suggesting an adipogenic and neuroregulatory role of NLC in cows with SCK. Overall, we identified a novel cell type in AT of dairy cows, the transcriptional profile of which changes with ketosis, playing a potential regulatory role on AT metabolism. Modulation of NLC could be potentially targeted to alleviate AT dysfunction.

Key Words: postpartum, adipose, transcriptomics

1216 Skeletal muscle transcriptome changes associated with ketosis in postpartum dairy cows. L. M. Avila-Granados*, N. Taechachokevivat, and R. C. Neves, *Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Purdue University, West Lafayette, IN*.

This abstract is part of the ADSA-Graduate Student Competition: Production (MS Oral Semifinalists).

Excessive body fat mobilization during the transition period overwhelms the liver's fatty acid (FA) oxidative capacity, leading to increased ketone body production and ketosis in dairy cows. Skeletal muscle (SM) has been shown to uptake and oxidize FA via mitochondrial β -oxidation and may play a crucial role in counteracting the hepatic FA overload. However, the role of SM in the pathogenesis of ketosis remains unclear. Our objectives were to investigate transcriptomic alterations in the SM of postpartum dairy cows diagnosed with ketosis to identify genes and pathways potentially involved in the pathogenesis of the disease. Fifteen ketotic and 15 healthy controls within 7 d in milk (DIM) were classified based on their blood β -hydroxybutyrate (BHB) concentrations (ketosis: BHB ≥ 1.3 mmol/L associated with clinical signs; control: BHB ≤ 1.1 mmol/L). On the day of enrollment, a skeletal muscle biopsy from the external oblique muscle was performed and later processed for RNA sequencing. Data analysis was performed using DESeq2 v1.44.0 and ClusterProfiler v4.2.2. Gene expression analysis identified 1,481 differentially expressed genes (DEGs) ($\log_2\text{FC} \geq 1$ or ≤ -1 ; p -adj ≤ 0.05), with 635 genes upregulated and 846 genes downregulated in the ketosis group compared with controls. Gene ontology and KEGG enrichment analyses suggested upregulation in multiple gene functions and biological processes during ketosis, including muscle contraction, cytoskeleton, protein digestion and absorption, and biosynthesis of amino acids (p -adj ≤ 0.05). Notably, genes involved in lipid metabolism and mitochondrial activity, such as sarcolipin ($\log_2\text{FC} = -2.23$, p -adj < 0.001), perilipin 5 ($\log_2\text{FC} = -1.00$, p -adj < 0.001), and pyruvate dehydrogenase kinase 4

(PDK4) (Log2 FC = 1.75, p-adj <0.000) were differentially expressed between groups. Although these findings suggest the involvement of SM with ketosis, further research is needed to determine whether SM dysfunction contributes to the onset and/or progression of the disease.

Key Words: ketosis, skeletal muscle, RNA sequencing

1217 Palmitic and oleic acids restore mitochondrial respiration in bovine adipocytes during a negative energy challenge. U. Abou-Rjeileh*, A. L. Lock, and G. A. Contreras, *Michigan State University, East Lansing, MI.*

Increased energy demand during the transition period in dairy cows often leads to excessive lipolysis, mitochondrial dysfunction, and elevated reactive oxygen species (ROS) in adipose tissue (AT). In humans, palmitic acid (PA) is linked to mitochondrial stress, whereas oleic acid (OA) supports normal function. However, their direct effect on mitochondrial function in dairy cows remains unknown. This study aimed to investigate the effects of lipolysis, PA, and OA on mitochondrial respiration and ROS production in bovine adipocytes. Pre-adipocytes were isolated from subcutaneous AT of nonlactating, non-gestating dairy cows (n = 3–6) and induced to differentiate. Mature adipocytes were cultured in standard differentiation media, supplemented with PA, OA, or PA-OA mixture (60% PA, 40% OA) at 300 μ M. On d 7, lipolysis was stimulated with 1 μ M isoproterenol (ISO) for 0.5, 2, and 4 h, with a basal (BAS) condition (no ISO) as control. Mitochondrial respiration was assessed using a Seahorse XF Analyzer. The ROS were quantified using the OxiSelect Intracellular ROS Kit. The statistical model included cow as a random effect, and treatment, ISO, and their interaction as fixed effects. Overall, basal, maximal, and ATP-linked respiration decreased post-lipolysis, with the greatest drop at 2 h ($P < 0.01$). By 4 h, respiration recovered to BAS levels. Similarly, spare respiratory capacity was lower at 0.5 and 2 h vs. 4 h and BAS ($P = 0.05$). The OA restored basal respiration, whereas PA and OA recovered ATP production ($P < 0.01$). Under BAS, PA-OA increased ATP production compared with other treatments ($P = 0.05$). Fatty acid treatments had no effect on ROS levels under BAS ($P > 0.37$). Lipolysis increased ROS at 0.5 and 2 h compared with BAS and 4 h ($P < 0.01$). However, PA-OA reduced ROS at 0.5 h post-lipolysis ($P < 0.01$). By 4 h, both PA and PA-OA lowered ROS production ($P < 0.01$). Our results suggest that PA and OA support mitochondrial function and reduce oxidative stress in adipocytes during a period of negative energy balance. This offers insights into the potential for enhancing metabolic health in dairy cows during the transition and early lactation periods.

Key Words: metabolic stress, fat supplementation

1218 Immunity at the crossroads of cellular metabolism: Navigating T helper shifts in the periparturient period of dairy cows. T. Fernandez-Wallace*, U. Arshad, M. Mancheno, M. Cid de la Paz, G. Praisler, T. France, H. M. White, and L. Rostoll-Cangiano, *Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

This abstract is part of the ADSA-Graduate Student Competition: Production (MS Oral Semifinalists).

The periparturient period in dairy cows brings major metabolic and physiological shifts, increasing disease susceptibility and immune dysfunction. The contribution of T helper (CD4⁺) cells to this altered state remains poorly understood. This study evaluates the in vitro metabolic function and proliferation capacity of CD4⁺ cells during the periparturient period. Holstein cows (n = 18) with a mean $\pm$ SD lactation of

4.16 $\pm$ 1.34 were enrolled and blocked based on expected calving date. Blood samples were collected at –28 (dry period), +3 (postpartum), and +28 (early lactation) days relative to calving (DRTC). Peripheral blood mononuclear cells were isolated and CD4⁺ T cells were obtained using magnetic sorting. A Seahorse XF HS Mini Analyzer was used to assess the metabolic function of CD4⁺ T cells through a T cell metabolic persistence assay, measuring glycolysis and oxidative phosphorylation via extracellular acidification and oxygen consumption rates. Cells were treated with assay medium (resting) or phorbol 12-myristate 13-acetate and ionomycin (stimulated). For proliferation capacity, CD4⁺ T cells were incubated with concanavalin A for 72 h and tracked using Cell Trace in Flow Cytometry. Data were analyzed in SAS 9.4 using a linear mixed-effect model with repeated measures. The statistical model included the fixed effects of DRTC, time of assay, morbidity, their interactions and random effect of block and cow. Cellular glycolysis was greater ($P < 0.01$) in CD4⁺ T cells during the postpartum and early lactation period as well as oxidative phosphorylation capacity ($P < 0.05$) in both resting and stimulated cells. The proliferation capacity of CD4⁺ T cells according to their expansion index was higher ($P < 0.05$) at postpartum compared with the dry period (–28 = 1.91, +3 = 2.65, and +28 = 2.38 $\pm$ 0.19). These findings suggest that CD4⁺ T cells metabolism and proliferation capacity are upregulated after calving. Metabolism of T helper cells appears to be affected by the physiological changes that occur after calving, which may impact the effectiveness of immune responses during the periparturient period.

Key Words: health, metabolism, transition cow

2809 Impact of energetic states on functional and transcriptional responses of bovine circulating neutrophils to in vitro lipopolysaccharide challenge. L. Xie*, S. M. Chandrappa^{1,2}, S. G. Andueza¹, H. Sadeghi^{1,3}, P. Karis^{1,4}, M. H. Rashid¹, M. Niazi¹, Y. Fan⁵, K. X. Qiao¹, Q. Dong⁶, L. Vincenti², A. Ricci², G. Opsomer¹, and O. B. Pascottini^{1,7}, ¹Department of Internal Medicine, Reproduction and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Ghent, Belgium, ²Department of Veterinary Sciences, University of Turin, Grugliasco, Turin, Italy, ³College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, ⁴A Chair of Animal Nutrition, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, Estonia, ⁵Department of Veterinary and Biosciences, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Ghent, Belgium, ⁶College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi, P. R. of China, ⁷School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland.

Polymorphonuclear neutrophils (PMN) are pivotal in the innate immune response of dairy cows. Their functionality (phagocytosis [PC] and oxidative burst [OB]) and gene expression patterns can be modulated by varying energetic conditions. Energetic fluctuations are evident in periparturient dairy cows, when distinct levels of negative energy balance (NEB) can trigger PMN dysfunction. This study investigated the impact of different energetic conditions on functionalities and transcriptomic landscape of PMN subjected to a lipopolysaccharide (LPS) challenge in vitro. Circulating PMN isolated from puberal nulliparous heifers (n = 4) were suspended in cell culture medium and supplemented with glucose (1.5 and 3 mM to mimic hypo- and normo-glycemia, respectively) and with 1.2 mM nonesterified fatty acids (NEFA; to mimic NEB peak). Control PMN suspension with no glucose nor NEFA supplementation were incubated alongside. After 3 h of incubation under different energetic conditions, PMN were challenged with 50 μ g/mL LPS for 1 h to

mimic infection. At 0 and 3 h of incubation, the PMN viability (viable, apoptotic, or necrotic) and functionalities (PC and OB) were measured via flow cytometry. After the LPS challenge, total RNA of PMN was extracted and sequenced on an Illumina platform. The effects of glucose and NEFA in the culture medium on PMN viability and functional parameters were fitted in mixed linear regression models, using the replicate as a random effect. The analysis of differentially expressed (DE) genes was performed by edgeR. In the absence of glucose, NEFA supplementation increased PMN apoptosis and OB capacity compared with no NEFA supplementation. Transcriptomic analysis showed that PMN exposed to 3 mM glucose plus NEFA exhibited downregulation of oxidative phosphorylation and reactive oxygen species pathways compared with 3 mM glucose alone (no NEFA), suggesting immunosuppression. Under NEFA supplementation, PMN exposed to 1.5 mM glucose showed upregulation of energy metabolism and immune signaling pathways when compared with 3 mM glucose, indicating a compensatory response to metabolic stress (presence of NEFA) when glucose is low. These results provide insights into how metabolic conditions during the transition period induce innate immune dysfunction in dairy cows.

Key Words: transition period, immune function, RNA sequencing

1220 Effects of PBMC-conditioned media on visceral adipose tissue endothelial cell transcriptome and proliferation in postpartum dairy cows with ketosis. H. Ford^{*1}, B. Sparks¹, V. Machado¹, O. Nelson¹, N. Beneduzi¹, O. Benitez Rojas^{1,2}, and C. Strieder-Barboza^{1,2}, ¹Texas Tech University, Lubbock, TX, ²School of Veterinary Medicine, Texas Tech University, Amarillo, TX.

Our objective was to evaluate the effect of PBMC-conditioned media on the transcriptional and functional characteristics of adipose endothelial cells (EC) in dairy cows with and without ketosis. Blood for PBMC isolation and omentum adipose samples (laparotomy) for EC isolation via flow cytometry sorting (CD31⁺/CD45⁻) were collected from 6 ketotic (KET; BHB ≥ 1.2 mmol/L) and 6 non-ketotic (NK; BHB ≤ 0.8 mmol/L) dairy cows (7 ± 1.7 DIM). Conditioned media (CM) from 1×10^6 PBMCs were collected after 24 h incubation in 10% FBS-DMEM/F12. Isolated EC were treated with CM from autologous PBMC (same cow) or pooled CM from the opposite treatment (KET or NK) for 48 h before RNA extraction for sequencing (Illumina NovaSeq X Plus) and for 7 d for proliferation analysis via daily cell counts using Cytation 5 (Agilent) imaging. Transcriptome data were analyzed via DESeq2 and clusterProfiler in RStudio, v4.4, and proliferation data via linear regression in SAS (v9.4) with treatment and day as the fixed effects and significance declared at $P \leq 0.05$. We identified 229 DEG ($\log_2\text{FoldChange} > |0.2|$) and adjusted P -value ≤ 0.05) in NK EC vs. KET EC treated with autologous CM. Functional analysis of this comparison revealed activated pathways of EC proliferation and *WNT* signaling, and suppressed pathways linked to cell adhesion and blood vessel morphology. There was a minimal effect of CM on KET EC transcriptome, with only *ADGRL3* expression increased between KET EC treated with autologous KET CM vs. pooled NK CM. In contrast, there were 212 DEG (93 up, 119 down) in NK EC treated with NK CM vs. pooled KET CM, with activation of lipid handling and hormone signaling pathways when treated with KET CM. In vitro, differences in EC proliferation were minimal, with only tendencies on d 6 ($P = 0.06$) and 7 ($P = 0.07$) for reduced proliferation of NK EC treated with pooled KET CM vs. autologous NK EC. There was no effect of any CM on KET EC proliferation. Together, our results suggest a pro-angiogenic profile of KET EC vs. NK EC with the KET PBMC secretome promoting lipid metabolism and immune signaling in adipose EC, while also potentially impairing EC proliferative function.

Key Words: adipose, ketosis, endothelial

1221 Temporal changes and predictive value of daily rumination time preceding left displacement of the abomasum (LDA) in Holstein cows. A. Mirzaei^{*1}, R. Bisinotto¹, R. C. Chebel^{1,2}, and P. Peixoto¹, ¹Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL, ²Department of Animal Sciences, University of Florida, Gainesville, FL.

Objectives were to characterize daily rumination time using automated monitoring devices (AMD) ± 7 d relative to LDA diagnosis (d 0), and to evaluate its predictive value for early LDA diagnosis. Cows from a single farm diagnosed with LDA ($n = 460$) from October 2021 to June 2023 were matched with controls ($n = 1,811$) based on lactation number and calving date. Cows flagged by AMD alerts were evaluated by percussive auscultation followed by laparotomy for LDA diagnosis, which was corrected by omentopexy. Continuous, binary, and test characteristics variables were analyzed by generalized linear mixed models, multivariable logistic regression, and ROC analyses. Daily rumination (LSM $\pm$ SEM) was lower ($P < 0.001$) for LDA cows from d -7 to d -1 (375.2 ± 8.3 vs. 142.8 ± 8.0 min/d) vs. control group (444.1 ± 4.1 vs. 487.3 ± 3.9 min/d) and, despite a sharp increase after correction, remained lower ($P < 0.001$) for LDA cows from d 0 to d 7 (169.5 ± 8.0 vs. 518.5 ± 7.0 min/d) vs. controls (510.6 ± 3.9 vs. 557.9 ± 3.4 min/d). Twinning, metritis, mastitis, ketosis, and indigestion were identified as risk factors ($P \leq 0.04$) for LDA. Rumination time coefficient of variation (CV; 95% CI) from d -7 to 0 was greater ($P < 0.001$) for LDA (0.50; 0.47–0.54) compared with control cows (0.19; 0.18–0.20). Greater CV was also associated ($P \leq 0.07$) with increasing parity, metritis, ketosis, indigestion, and 2-way interactions between LDA and ketosis and between LDA and indigestion (i.e., indigestion and ketosis were associated with greater CV in controls, which was further increased in LDA cows). Sensitivity (Se.) of daily rumination to identify cows with LDA on d 0 ranged from 0.53 to 0.66 (d -7 to -4), increasing to 0.73 (d -3), 0.77 (d -2), and 0.93 (d -1). Specificity (Sp.) ranged from 0.59 to 0.69 (d -7 to -3), increasing to 0.80 (d -2) and 0.88 (d -1). Combination of rumination time with difference and ratio of change from previous day improved Sp. (0.85–0.96) at the expense of Se. (0.19–0.56). Changes in rumination time are associated with LDA and application of daily rumination time have the potential to hasten LDA diagnosis.

Key Words: wearable sensor, precision farming

1222 Rumination during the first week postpartum changed the effects of hyperketonemia on performance of dairy cows. C. Florentino^{*}, A. Ravelo, F. Pena-Mosca, D. Moreira, M. Ruch, E. Shepley, and L. Caixeta, University of Minnesota, Saint Paul, MN.

Around calving, dairy cattle undergo a metabolic shift to support early lactation. Leading to adipose tissue breakdown and increases blood ketone bodies, a disorder defined as hyperketonemia (HYK). While HYK's negative impact on milk yield (MY) is well documented, it remains unknown whether rumination behavior modifies the HYK-MY association. Our objective was to evaluate whether rumination recovery during the first week postpartum changed the association between HYK and MY across 300 DIM. Cows in 2 different herds in Minnesota were enrolled in this study. All cows calving between March of 2020 to February of 2022 were eligible. Cow side tests were performed to quantify β -hydroxybutyrate (BHB) in the blood once between 2 and 9 DIM. Cows with BHB ≥ 1.2 mmol/L were classified as HYK+ ($n = 108$), and those with BHB < 1.2 mmol/L as HYK- ($n = 824$). Rumination behavior was recorded during the first week postpartum, and cows were retrospectively assigned to a rumination recovery group (RRG) based on the average daily change in rumination time between 2 and 8 DIM. Rumination recovery rate was calculated blocked by herd and

parity (1st, 2nd, >3rd). Milk production data were obtained from farm herd software. Cows were categorized into 3 RRG: the top 25% were classified as high-speed (HS; 42.0 ± 17.2 min/d, $n = 232$), the middle 50% as medium-speed (MS; 23.2 ± 7.4 min/d, $n = 463$), and the bottom 25% as low-speed (LS; 1.6 ± 11.3 min/d, $n = 237$). Statistical analyses were performed using multiple linear regression models with mixed effects. Fixed effects included RRG, HYK status, DIM, parity, herd, cows that were treated for HYK, average rumination time on the week before calving, and an interaction term between RRG and HYK. Cows

were added as a random effect in all models. Among HS cows, HYK+ cows produced 3.2 kg/d (95% CI: 0.1–6.2 kg/d) more milk and 3.1 kg/d (95% CI: –0.1–6.3 kg/d) more FCM than HYK– cows across 300 DIM. No differences were observed in LS and MS groups. Findings suggest that higher rumination recovery rates are linked to increased MY and FCM in HYK+ cows.

Key Words: milk production, ketosis, daily behavior

Breeding and Genetics Symposium: Joint ADSA-Interbull Session— Creating Carbon-Friendly Cows and Leveraging Omics to Improve the Sustainability of Dairy Production

1223 Breeding for more sustainable dairy cows. F. Peñagari-cano*, *University of Wisconsin–Madison, Madison, WI.*

Sustainability, defined as meeting the needs of the present without compromising the ability of future generations to meet their own needs, is the balance between the economy, society, and the environment. Achieving a sustainable dairy farming is paramount to address the major challenges facing humankind, including food security, energy use, biodiversity, climate change, and the environmental footprint of human activity. Three components play a crucial role in dairy farm sustainability: feed efficiency, methane emissions, and resilience. Feed accounts for more than 50% of total production costs, and hence, improving feed efficiency increases farm profitability and reduces the environmental impact of milk production. Enteric methane is a major source of greenhouse gas emissions from dairy farming. Methane also represents a loss of gross energy intake, energy that could otherwise be available for milk production. Therefore, reducing methane emissions from dairy cattle provides multiple benefits, including reducing the environmental impact of dairy farming and improving production efficiency. Resilience can be defined as the capacity to maintain performance or bounce back to normal functioning after a perturbation. These perturbations are associated with any factor that can cause a dairy cow to be stressed, such as diseases, temperature changes, or management alterations. Improving cow resilience is critical to safeguard the long-term sustainability of the dairy industry. Overall, the success of dairy farming can be seen as the ability of cows to produce milk, while minimizing environmental impact, as well as enhancing cows' health and welfare, within socially acceptable standards. Genetic selection is arguably a critical tool to achieve these sustainable goals.

Key Words: feed efficiency, methane emission, resilience

1224 Advancing dairy sustainability through feed-efficient genetics and genomics: Research insights and applications. J. R. Johnson*, N. Deeb, P. Khanal, and P. Ross, *ST Genetics, Navasota, TX.*

As the dairy industry faces increasing pressure to optimize resource use and minimize its environmental footprint, the need for innovative, scientifically sound, and economically feasible solutions to enhance feed efficiency is more urgent than ever. Genetic selection, powered by recent advances in genomics, has already transformed breeding to enhance productivity, reproductive efficiency, and longevity. Now, as a result of significant private and public funding to capture individual animal feed intake at scale, genetic selection indices for feed efficiency—with particular emphasis on residual feed intake (RFI)—have emerged, offering a lasting and cumulative solution to enhance feed efficiency. Residual feed intake, which accounts for variation in feed intake independent of maintenance and productivity, enables producers to identify animals that use feed more efficiently, reducing feed costs and carbon emissions without compromising productivity. Moreover, RFI selection can be integrated with other traits to improve feed efficiency while simultaneously enhancing key attributes such as milk or meat production. With the rapid adoption of RFI in current selection programs, the industry is entering a new era of dairy farming where sustainability and profitability go hand in hand. This presentation will draw on extensive progeny data from a large-scale research program alongside commercial farm data

to explore collaborative research findings and discuss implications of RFI selection on animal health, productivity, and sustainability. By examining on-farm data we will provide insights into how RFI selection is being applied to Holstein heifers and cows and how producers are leveraging genetic tools to optimize feed efficiency, reduce feed cost, and lower the carbon footprint of their operations. The findings will also demonstrate how these efforts contribute to broader sustainability goals in dairy production and play a crucial role in mitigating agriculture's environmental impact.

Key Words: feed efficiency, residual feed intake, sustainability

1225 Sustainable breeding for feed-efficient cows with lower methane emissions. B. Heringstad*^{1,2} and K. A. Bakke², ¹*Department of Animal and Aquacultural Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, Aas, Norway,* ²*Geno Breeding and A.I. Association, Aas, Norway.*

To meet future challenges and reduce climate impact according to national goals, we need to include feed efficiency and methane emissions in the dairy cattle breeding goals. Collecting phenotypes for these traits in dairy cattle is challenging, expensive, and requires significant time and labor. However, access to reliable phenotypes for the goal traits on a sufficient number of animals is crucial for success. Recording of individual feed intake and methane emissions is now established in research and commercial herds across many countries, and international initiatives and collaboration are in place to expand the reference populations for genomic evaluations. In Norway, large-scale phenotyping of methane emissions and feed intake of Norwegian Red cows is established in collaboration with 14 commercial dairy herds. All cows are genotyped and housed in freestalls with automatic milking systems. Equipment to measure individual roughage intake (<https://biocontrol.no>) and GreenFeed units (www.c-lockinc.com) for measuring methane emissions are in place. The growing database of high-quality phenotypes of key parameters makes it possible to increase knowledge and develop the tools needed for a successful implementation of breeding feed-efficient and climate-friendly cows. Results so far indicate substantial genetic variation for methane emission, roughage intake, dry matter intake, and different measures of feed efficiency in the Norwegian Red breed. The estimated heritability of daily methane, dry matter intake, and body weight ranged from 0.18 to 0.57 and the genetic correlations among these traits were between 0.66 and 0.81. Our project has also enabled new knowledge on genetic variation in the cow's response to methane-mitigating feed additives. More knowledge is needed on the genetic associations to important traits, and further research is required to examine which trait definitions would be better for successful implementation in the breeding program.

Key Words: environmental impact, feed efficiency, genetic parameter

1226 Development of a greenhouse gas index to reduce enteric emissions in Holsteins. F. Malchiodi*¹, C. Richardson², S. G. Narayana³, A. Fleming³, C. F. Baes⁴, and F. Miglior^{1,4}, ¹*Semex, Guelph, ON, Canada,* ²*AbacusBio, Edinburgh, United Kingdom,* ³*Lactanet, Guelph, ON, Canada,* ⁴*University of Guelph, Guelph, ON, Canada.*

Genetic selection offers a cost-effective, long-term strategy for reducing greenhouse gas (GHG) emissions in dairy cattle, enhancing sustainability with each generation. With a focus on genetic advancements in specific traits, such as methane production, selection indexes that integrate both economic performance and environmental impact can be developed. Several countries are incorporating emissions-related traits into national selection indexes, with successful adoption varying based on industry-specific objectives and constraints. Canada has introduced the world's first national methane breeding value system for dairy cattle (methane efficiency) based on enteric methane production adjusted for milk, fat, and protein yield. Successfully validated using both domestic and international data, this genomic evaluation has demonstrated its efficacy in accurately identifying genetic variation associated with methane reduction, confirming its potential as a valuable tool for miti-

gating emissions in dairy cattle. In addition to methane efficiency, traits related to body size, longevity, and feed efficiency have been identified as having independent effects on overall system-wide emissions. These traits have been incorporated into an overall sustainability index, which reduces emissions by 168 kg CO₂e per cow per year for each standard deviation increase in the index. Results suggest that the most effective breeding approach involves integrating a direct methane trait alongside increased emphasis on environmentally impactful traits. This sustainability-focused index was incorporated into the Lifetime Performance Index (LPI) in April 2025, reflecting the dairy industry's commitment to profitability, sustainability and environmental stewardship.

Key Words: sustainability, methane, genomic

Dairy Foods: Cheese and Chemistry

1227 Impact of baking temperatures and low-proteolytic rennets on extending the squeakiness shelf-life of Juustoleipä-style cheese curds. M. A. Becher^{*1}, S. Govindasamy-Lucey², J. J. Jaeggi², M. E. Johnson², B. G. Prochaska², G. N. Gnanateja¹, and J. A. Lucey^{1,2}, ¹University of Wisconsin–Madison, Madison, WI, ²Wisconsin Center for Dairy Research, Madison, WI.

Fresh cheese curds are a common snack cheese in Wisconsin and some other US cheesemaking regions. Curds are the result of the cheddaring and milling processes but are not pressed into a block. The squeaky sound, which occurs when the curd protein matrix slides across the teeth while chewing, is an important quality attribute and is greatly diminished after a few days of refrigerated storage. Another squeaky cheese, Juustoleipä (Juusto), is known to retain squeakiness for months. We hypothesized that the high baking temperature during Juusto manufacture inactivates proteolytic enzymes, which leads to higher levels of intact casein and elasticity during storage, thus retaining squeakiness. To test our hypotheses, we produced Juusto-style curds that were not acidified, were coagulated with 2 different low proteolytic rennets, and were baked to an internal temperature of 60°C (LB), 88°C (HB), or were not baked (NB). Squeakiness of curds was monitored for 60 d using frequency-based acoustic analyses, as well as with descriptive sensory analysis. Proteolysis was determined via pH 4.6 soluble N and capillary electrophoresis. Elasticity was determined via frequency sweep rheological analyses. Data were statistically analyzed using a split-plot design and Tukey testing with an α -level of $P < 0.05$. The rennet type did not greatly impact the properties of Juusto curds, but the HB temperature produced a browned exterior, reduced moisture content, reduced proteolysis, retained firmness, slightly altered the flavor (higher baked flavor), and retained squeakiness for a longer period than the NB and LB curds. The LB temperature also reduced proteolysis and retained squeakiness for a longer period than the NB curds ($P < 0.05$). The loss tangent (LT) values demonstrated that the HB curds retained a similar LT level at 2 and 60 d of storage, but the LT of the LB and NB curds increased during storage, indicating a loss of elasticity, with a corresponding loss of squeakiness. A reduction in proteolysis during storage ($P < 0.05$) helped retain elasticity and thus maintained squeakiness. Cheeses with high insoluble Ca levels and little proteolysis retained squeakiness for a longer refrigerated storage period.

Key Words: cheese curd, squeakiness

1228 A mechanistic description of microwave vacuum drying and puffing of cheese. B. Gong and C. I. Moraru^{*}, Cornell University, Ithaca, NY.

Microwave vacuum drying (MVD) has been shown to induce an expansion effect in cheese and thus can be used to create puffed dried cheese snacks. Here, we will discuss how process-related factors and product characteristics affect drying kinetics and the physical phenomena that control MVD puffing of cheese. Commercial low-moisture, part-skim mozzarella and mild Cheddar were cut into 1 cm cubes and processed at different vacuum levels (1.7–15.8 kPa) and microwave (MW) power levels (1–3 W/g) in a pilot-scale nutraREV 10 kW MVD unit (EnWave Corporation). The cheese tensile properties (Young's modulus) were measured using a TAXT2 texture analyzer, and rheological properties (G' , G'') and glass transition temperature (T_g) of the cheese of different moisture contents were measured using a strain controlled rheometer (TA Instruments), at different temperatures. T_g was mainly influenced

by vacuum level and was independent of MW power in the range of conditions tested. Tensile tests showed that at a certain temperature before being fully melted, designated as the “flow-point” T_f , the cheese became very soft and self-deformed. The drying kinetics were described by the semi-empirical Balbay-Sahin model ($R^2 > 0.99$). The moisture sorption isotherm and T_g at different moisture contents of the cheese matrix were coupled with the drying kinetics to generate the cheese state diagram. The state diagram showed that T_g can be correlated with the tensile properties during the expansion phase and the degree of puffing. At $T_g < T_f$ expansion increased with T_g , maximum expansion was observed at $T_g \approx T_f$, and collapse and/or spreading occurred at $T_g > T_f$. This work provides a guide for processors to predict cheese puffing and drying during MVD, allowing them to determine conditions that can be used to produce dried cheese snacks of desired structures and characteristics (i.e., puffs vs. crisps).

Key Words: cheese, microwave vacuum drying, puffing

1229 Thermal imaging: A cost-effective method for estimating the melting point of cheese. Z. S. Modi^{*} and P. Salunke, South Dakota State University, Brookings, SD.

Transition temperature (TT) is used to measure melting point using a rheometer, which measures the change of cheese from solid to viscous state as a function of temperature. This process takes a long time and requires specialized equipment, a laboratory, and trained personnel. It becomes a challenge for the salesperson to measure meltability on-site. Thermal infrared (IR) image camera and software analysis can be used to measure cheese's meltability and melting point. The study was designed to develop a method to estimate the melting point of cheese using a thermal IR camera, providing a more practical and cost-effective alternative to conventional rheological analysis. Commercial samples of cheeses, including natural cheeses and low-moisture, part-skim mozzarella, were procured from the market. The samples were analyzed for meltability using the tube melt test (TMT), modified Schreiber melt test (MSMT), rheological (G' , G'' , and TT), and texture profile analysis (TPA). The thermal IR image camera test method was developed, and the data were processed using software to determine the melting point. The experiment was replicated thrice using different lots of cheeses, and the data were statistically analyzed. The thermal image was captured after 1 min at room temperature following MSMT. The captured image was analyzed using the software, and the melting point from this image analysis was correlated with the rheological TT using correlation coefficient (R^2) to validate the reproducibility. The melt characteristics differed significantly ($P < 0.05$) according to cheese type. The TMT, MSMT, and hardness results varied according to the type of cheese, ranging from 51.51 to 111.67 mm, 596 to 1,370 mm², and 4,649 to 7,962 g. The TT obtained using a rheometer for cheeses varied between 51.88°C and 52.55°C, whereas the corresponding values from image analysis were between 52.08°C and 55.42°C. The R^2 values for the correlations ranged from 0.78 to 0.81, demonstrating a strong relationship between the rheological and image analysis methods. The required time to test the melting point was reduced from 75 to 8 min. The study demonstrated that a thermal IR method provides a practical, quick, and cost-effective approach for estimating the melting point in cheese compared with conventional rheological analysis and can be used on-site.

Key Words: thermal imaging, melting point, transition temperature

1230 Properties of A1/A2 and A2/A2 U.S. Cheddar cheese: Presence of β -casomorphin peptides. D. Daniloski^{1,2}, R. A. Ibáñez^{*2}, N. A. McCarthy³, T. Vasiljevic⁴, and J. A. Lucey², ¹*School of Chemical Engineering, The University of Queensland, Brisbane, QLD, Australia*, ²*Center for Dairy Research, University of Wisconsin–Madison, Madison, WI*, ³*Food Chemistry and Technology Department, Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland*, ⁴*Advanced Food Systems Research Unit, Institute for Sustainable Industries and Liveable Cities, Victoria University, Melbourne, VIC, Australia*.

Bovine β -casein (β -CN) is a common protein found in milk and has several phenotypes, distinguished based on their amino acid sequence. The 2 most studied variants, β -CN A1 and A2 differ at position 67 of the peptide chain, where β -CN A2 contains proline, while β -CN A1 has histidine. There has been significant research on the health implications of consuming dairy foods containing β -CN A1, but as of yet it is uncertain what role this protein has on any non-communicable diseases. This study aimed to investigate whether differences in β -CN polymorphism influence the properties of retail A1/A2 and A2/A2 Cheddar cheeses. A total of 26 cheese samples, comprising β -CN A1/A2 ($n = 16$) and A2/A2 ($n = 10$) from various maturation stages and individual producers, were sourced from the US market. The β -CN variant in each sample was confirmed via capillary electrophoresis. Additionally, the pH 4.6-soluble nitrogen, total free amino acids (TFAA), peptide profile and levels of selected β -casomorphins (BCMs) were analyzed to assess proteolysis. The samples met the compositional standards for Cheddar cheese outlined in the Code of Federal Regulations, although A2/A2 cheeses exhibited higher salt content ($P < 0.05$). All A1/A2 cheeses had significantly lower pH 4.6-soluble nitrogen and TFAA content than A2/A2 cheeses ($P < 0.05$). An important implication for Cheddar cheese production is that A2/A2 cheeses, which exhibit higher proteolysis, may require shorter aging times to achieve the desired flavor and texture compared with A1/A2 cheeses, which undergo slower maturation. Principal component analysis effectively distinguished A1/A2 and A2/A2 cheeses based on their peptide profiles. Notably, both cheese types contained a high amount of BCM-9 at levels of 0.36–127.11 mg/kg cheese, whereas BCM-7 was only detected in A1/A2 cheeses (0.11–9.75 mg/kg cheese). Overall, while A1/A2 and A2/A2 cheeses shared numerous compositional similarities, A2/A2 cheeses exhibited higher proteolysis and lacked the presence of BCM-7. These findings have important implications for Cheddar cheese production and should be carefully considered when manufacturing cheeses based on β -CN phenotype.

Key Words: cheese composition, β -caseins A1 and A2, β -casomorphin

1231 Flaky Provola dei Nebrodi PDO cheese: Effect of curd acidification on the flaking process. G. A. Calandra Checco^{*1}, S. Petriglieri², G. Licitra¹, M. J. Lo Faro¹, C. Caggia^{1,2}, G. Belvedere², G. Marino², C. Pisana¹, and M. Caccamo², ¹*University of Catania, Catania, Italy*, ²*CoRFiLaC, Ragusa, Italy*.

Provola dei Nebrodi PDO is a traditional stretched raw-milk cheese that develops internal flaking after months of aging. The factors influencing this phenomenon are still unknown, and flaking does not always occur. This study investigated how curd acidification at pH 4.8 (P4.8) and pH 5.2 (P5.2) affects flaking development during an aging period (AP) of 0, 60, 90, 120 d, assessing qualitative parameters. Curd was divided into 2 batches: one acidified to P5.2 reached in 3 h and the other to P4.8 reached in 9 h. In September, each batch was further divided into 14 3-kg Provola cheeses, one for each AP in both the P5.2 and P4.8 and aged at $14 \pm 1^\circ\text{C}$ and $80 \pm 1\%$ relative humidity. Microbiological analyses showed significantly higher lactic acid bacteria (LAB) and in particular entero-

cocci counts in P4.8 than in P5.2 ($P < 0.05$), while yeasts and molds exhibited no significant differences. Chemical analyses revealed distinct pH evolution, increasing from 5.06 to 5.32 in P4.8 and from 5.22 to 5.51 in P5.2 ($P < 0.05$). Fat on DM (FDM) significantly increased with AP ($P < 0.05$), reaching $49.75\% \pm 0.25$ in P4.8 and $51.08\% \pm 0.28$ in P5.2, from initial values of $42.33\% \pm 0.20$ and $43.82\% \pm 0.22$. Total protein on DM (TN/DM) followed a similar trend, increasing from $53.21\% \pm 0.30$ to $58.47\% \pm 0.28$ in P4.8 and from $51.94\% \pm 0.32$ to $57.12\% \pm 0.31$ in P5.2 ($P < 0.05$). Proteolytic activity (pH 4.6 soluble N) was greater in P4.8 during aging ($P < 0.05$), showing a more pronounced increase. Sensory analysis revealed significantly higher flaking intensity in P4.8 ($P < 0.001$), while P5.2 showed greater firmness and eye formation ($P < 0.05$) due to undesirable gas production (EFA). Confocal Laser Scanning Microscopy (CLSM) revealed significant differences ($P < 0.05$) in protein-lipid spatial distribution, highlighting the role of pre-stretching pH in microstructural and textural evolution. Statistical modeling included pH, AP, and their interaction as fixed effects, with significance at $P < 0.05$. These findings demonstrate that lower pre-stretching P4.8 enhances flaking, increases proteolysis, while reducing excessive EFA, improving PDO cheese production through controlled curd acidification.

Key Words: curd acidification, flaking, proteolysis

1232 Understanding factors affecting stickiness of commercial cheese slices. A. Crumpley^{*} and P. Sharma, *Utah State University, Logan, UT*.

Cheese is a growing industry in the United States, with sliced cheese among the most popular forms of cheese available at the retail level. Stickiness of these slices in retail format is a common problem, leading to slice breakage and a decrease in consumer convenience and product value. Stickiness in cheese may be influenced by many factors, including production conditions, such as degree and rate of acidification; packaging and storage conditions; and composition. The addition of emulsifying salts to processed cheeses can also affect the sticky properties of these products. The objective of this research was to identify the effects of cheese composition, temperature abuse, and contact material on tack properties of cheese. Commercial samples of 5 sliced cheese varieties were obtained from a local retailer. Cheese varieties included Cheddar, mozzarella, Havarti, Pepper Jack, and processed cheese. Composition tests (moisture, protein, salt, and fat) were conducted in triplicate for all samples. Tack force and tack energy were measured for all samples using a TA.XT plus texture analyzer with acrylic TA-4 probe at 5°C . The effect of surface modification on the tack properties of cheese was studied at 5°C for Cheddar and mozzarella samples using TA.XT plus texture analyzer with a bare acrylic TA-4 probe, cheese surface attached to TA-4 probe, and a bare stainless-steel TA-30 probe. The modified TA-4 probe had cheese adhered to the end of the acrylic TA-4 probe to measure tack force and tack energy at a cheese-cheese interface. The impact of temperature abuse was tested by allowing sliced cheese samples to rest at 21°C for ~ 3 h, then refrigerating sample for ~ 2 h before measuring for tack. For each tack testing 6 replicates were used. Among all cheese types, protein content and protein to moisture ratio were positively correlated with tack force ($r > 0.85$, $r > 0.75$) and tack energy ($r > 0.95$, $r > 0.79$), suggesting that higher protein cheeses may be stickier than those with lower protein content. This could be due to the fact that higher protein may also bring a little bit of moisture causing adherence to the probe. Cheese-cheese adhesion forces (23.46 ± 0.8423 N) for Cheddar cheese were less compared with Cheese-acrylic surface probe (25.86 ± 0.8829 N) and cheese-stainless surface (26.49 ± 0.8442 N). Additionally, temperature abused samples (24.17 ± 0.6739 N) showed lower tack force as compared with the normal samples ($25.86 \pm$

Table 1 (Abstr. 1233). Results of study

Validation	Summary of RMSEP values			Summary of MD values		
	Average			Average		
	NIR H	NIR NH	MIR	NIR H	NIR NH	MIR
Fat	0.013	0.030	0.016	0.014	-0.006	-0.008
Lactose	0.028	0.029	0.007	0.041	0.024	0.003
Protein	0.008	0.015	0.016	0.004	0.002	-0.015
Solids	0.020	0.036	0.023	-0.003	-0.014	-0.020

0.8829 N) when tested at the same temperature suggesting the release of fat on the surface plays some role in the tackiness.

Key Words: cheese slice, stickiness, tack test

1233 Improved partial least squares prediction models for homogenized and un-homogenized milk using transmittance near-infrared spectroscopy. A. Spillane* and D. M. Barbano, *Cornell University, Ithaca, NY.*

Near-infrared signals (NIR) are weaker than mid-infrared (MIR) and therefore typical NIR has much higher root mean error of prediction (RMSEP) values than MIR. Our objective was to determine if the use of milk spectra from a combination of modified milks and individual farm milks with all-laboratory mean (n = 8 laboratories) reference chemistry would produce NIR partial least squares (PLS) prediction models that would have smaller RMSEP for farm milks than models developed from only from farm spectra of individual farm milks. For the modeling, a population of spectra from 56 farm milks (from different regions of the US over a 4-mo period) and 42 modified milks (3 independent sets of 14 samples produced over 3 mo). This approach has worked well for PLS model development in mid infrared (MIR). The NIR milk analyzer used had 1 cm flow through cuvette with an in-line homogenizer that could be in the flow system before the cuvette or bypassed. Two PLS NIR models were developed, one for homogenized and one for non-homogenized milk. The external validation performance of the 2 NIR Models a fat, true protein, lactose and total solids models were compared with performance of MIR models. Validation was done with 48 individual farm milks, from different regions of the US over a 3-mo period, that had all-laboratory mean (n = 8) chemistry reference chemistry values. All NIR and MIR models were slope and intercept adjusted with the same set of modified milk calibration samples each month before analysis of the validation samples. The RMSEP and MD values for validation are shown in Table 1. Our RMSEP for NIR are better than values reported in the literature for milk analysis, but were still not as good as MIR. The mean difference on validation for the NIR for lactose was larger than MIR. Further work is needed to improve the NIR lactose and total solids models.

Key Words: near infrared, milk component, transmittance

Table 1 (Abstr. 1234). Analytical and statistical parameters

Item	Linearity (R ²)	LOD (mg/L)	Sensitivity (per mg)	Precision (RSD %)
LC-UVVIS	0.998	7.5	1,391.6 ± 51.7	<3% (n = 10)
LC-MS	0.999	12	390.4 ± 11.6	<6% (n = 6)
Enzyme kit	0.999	2.96	736.5 ± 2.9	<2.5% (n = 3)

1234 Determining milk sugar content in acid whey using a single Quad LC-MS system in comparison with UV-VISis detection and enzyme-based assay technique. A. J. Stout*, M. D. Asaduzzaman, J. Pico-Carbajo, and P. Sharma, *Utah State University, Logan, UT.*

Milk sugar determination on amide columns is hard due to polarity and ionization. They have been analyzed on LC with RID detectors, but RID is an additional purchase. A method for quantifying milk sugars was developed by derivatizing them with a chromophore to separate on C18 column and detect with UV-VIS. Enzyme kits are convenient, but limit of detection is less accurate than UV-VIS or MS detectors. In this study, linearity, selectivity, sensitivity, repeatability/LOD, accuracy, and precision of methods were compared in an acidic medium. Milk sugar solutions of 0 to 500 ppm mg/L were prepared, then were derivatized with 1-phenyl-3-methyl-5-pyrazolone (PMP) at 70°C for 2 h, extracted with solid phase extraction, then filtered in 0.2-µm nylon filters. The LC analysis was done with a Shimadzu Nexcol C18 5 µm, 3.0 × 50 mm column, UV-VIS detector at 248 nm with ammonium acetate mobile phase (7.7 g/L, pH of 5.51) and acetonitrile in gradient mode. Flow rate: 0.3 mL/min, column oven: 30°C. The LC-MS method, MP was acetonitrile: water: methanol (90:6:4) with 0.05% (vol/vol) dimethylamine, 500 mg/L ppm guanidine hydrochloride, filtered through a 0.2-µm PTFE. Column oven: 90°C, an isocratic flow rate: 0.6 mL/min, and injection volume: 1 µL. MS parameters: desolation line: 250°C, heat block: 400°C, nebulizing gas flow 1.5 L/min, and drying gas flow 15 L/min. Selected ion monitoring using negative ion mode was applied at 377 and 379 m/z. Enzyme assay followed standard protocol. All analyses were done in triplicate. Methods were validated for parameters mentioned above. Calibration curve constructed in linear dynamic range (0–500 ppm) of the lactose standard. Table 1 shows analytical performance data. Good linearity (R² = 0.999) was observed for both methods. Selectivity was tested with a blank sample. No interference occurred at retention time (11.2 min) of lactose standard. Better repeatability (RSD < 3%) LOD (7.5 mg/L) was observed for LC-UVVIS than LC-MS method with LOD being lower for the enzyme kit. This research will benefit analysis of acid whey lactose on a C18 column.

Key Words: refractive index detector (RID), limit of detection (LOD), relative standard deviation (RSD), liquid chromatography-mass spectrometry (LC-MS), ultraviolet visible (UVVis)

Dairy Management Inc. Symposium: Advances in Membrane-Filtration Ingredients—Technology and Applications

1235 Protein ingredients for high-protein beverages: Insights and applications. D. M. Barbano¹ and M. A. Drake^{*2}, ¹*Cornell University, Ithaca, NY*, ²*North Carolina State University, Raleigh, NC*.

Milk proteins have unique flavor, functional, and nutritional properties that align with consumer desires and provide opportunities for innovation. A simple ingredients deck is desirable to today's consumer, and dairy protein functionality can be utilized to exclude undesirable ingredients (stabilizers, thickeners) from protein beverages. Heat stability (protein aggregation and gelation) remains an ongoing challenge for both extended shelf life and shelf stable milk and milk protein beverages. Removal of lactose and low molecular weight soluble solids increases heat stability of milk proteins. Consumer perception of protein beverages and the role of milk components on protein beverage stability will be addressed.

Key Words: consumer perception, protein beverage, heat stability

1236 Innovations in protein ingredients for high-protein yogurt manufacturing. J. K. Amamcharla*, *University of Minnesota, Minneapolis, MN*.

Milk-derived ingredients such as milk protein concentrate (MPC) and isolates are widely utilized for their nutritional and functional benefits in various dairy and food products. These ingredients provide functional benefits such as thickening, gelling, and emulsification. These properties are influenced by protein concentration and interactions between proteins, including casein-casein, casein-whey protein, and whey protein-whey protein interactions. Whey proteins, which make up approximately 20% of the total proteins in milk, are globular proteins highly susceptible to denaturation followed by aggregation during processing. This denaturation exposes sulfhydryl and/or disulfide residues, leading to intra- or intermolecular crosslinking, which can alter functionality. By controlling these interactions, the final functionality of MPC can be customized. In this study, selective modifications, such as fibrillation or pre-aggregation of whey proteins, were applied to produce MPC suitable for a wide range of applications. Ingredients like MPC and NDM, in which whey proteins were selectively converted to fibrils or soluble aggregates, were produced and characterized in terms of acid gelation and other functional characteristics. The MPC with fibrillated whey proteins demonstrated significantly higher viscosity, and acid gels made with this ingredient resulted in firmer gels compared with control MPC. In a separate study, nonfat dry milk (NDM) containing whey protein fibrils was developed using microfiltration and ultrafiltration to produce fibrillated skim milk, which was then spray-dried to create fibrillated NDM. Reconstituted fibrillated NDM also showed improved viscosity, emulsification, and foaming capacity. These findings suggest that selective modifications of whey proteins can enhance the functionality of milk protein powders, making them suitable for various formulations. These whey protein-functionalized ingredients can reduce the need for non-dairy additives and can be classified as clean-label ingredients.

Key Words: ingredient functionality, acid gel, protein interaction

1237 Membrane filtration in cheese production. S. Govindasamy-Lucey*, *Center for Dairy Research, University of Wisconsin-Madison, Madison, WI*.

There is growing interest in manufacturing cheese from concentrated milk as this increases plant productivity and output. Low concentration factor ultrafiltration (LCF-UF) has been extensively used by the cheese industry, to standardize protein content and reduce seasonal variations in composition and cheese properties/yield. When protein levels in cheese milk are <4%, only minor modifications in the cheesemaking recipe are needed. Low concentration factor microfiltration (LCF-MF) is also becoming popular and has similar benefits to LCF-UF but also removes whey (serum) proteins before cheesemaking. This allows the cheese milk to be standardized to a specific casein (CN) content while removing whey proteins as milk-derived whey (value-added ingredient). Using MF to deplete most whey proteins in cheesemilk does not impact the nutritional profile and more characteristic cheese ripening is observed. We have been exploring cheese milks with CN levels between 4% and 10% made with both UF and MF. Use of higher protein levels requires modifications to address the rapid rennet coagulation process, such as reducing the coagulation temperature and precise selection of the optimum cutting time (as well as a faster cutting cycle). Membrane filtration results in milk that has a higher proportion of insoluble calcium as a percentage of total calcium. Preacidification with different types of acids, or carbon dioxide, can help to partially demineralize milk, along with use of a lower pH at whey drainage. However, fat losses after cutting can prove to be a significant issue with highly concentrated milk. Fat losses in low-moisture, part-skim mozzarella cheeses made from high CN cheesemilk (~9%–10% CN) can be decreased by use of preacidification and low pressure homogenization of the cheesemilk, as well as by using a pressed-block process instead of pasta-filata style (to avoid further fat losses in the cooker/stretcher). While concerns remain regarding the impact on cheese quality by the use of concentrated milk, these approaches will become more popular for commodity type cheeses due to the increased yield, more consistent quality, as well as greatly increased factory output.

Key Words: ultrafiltration, microfiltration, mozzarella cheese

1238 Novel membranes for valorizing dairy coproduct streams. J. Hestekin*, *College of Engineering, University of Arkansas, Fayetteville, AR*.

Dairy processing relies heavily on membranes, but the challenge of fouling necessitates harsh cleaning methods. This project aims to overcome this hurdle by employing an innovative and eco-friendly approach to develop specialized zwitterionic membranes. These membranes will be utilized for producing milk protein concentrates and potentially fractionate dairy proteins. Zwitterionic materials, known for their anti-fouling properties, have shown promise in various applications. However, many existing materials are not suitable for industries involving human consumption, such as food and pharmaceuticals. In this project, we will synthesize and test a nanocellulose zwitterionic membrane for ultrafiltration performance. The utilization of biocompatible zwitterionic materials will address the pervasive issue of protein fouling, a significant challenge in dairy product processing. The nanocellulosic membrane has been shown to make "slit" membranes so may be able to have better fractionation capabilities than polysulfone membranes currently used in the dairy industry. This innovation has the potential to revolutionize protein extraction from dairy products, ensuring higher efficiency and reduced waste.

1239 Protein interactions in whey protein phospholipid concentrate manufacturing. G. Ozturk*, *University of Wisconsin–Madison, Madison, WI.*

Whey protein phospholipid concentrate (WPPC) is a by-product of whey protein isolate production and serves as a source of various bioactive compounds that benefit human health. Our recent findings demonstrated that WPPC contains elevated levels of milk fat globule membrane (MFGM) proteins, which account for 23% of the total relative protein; phospholipids constitute 20% of the total fatty acid pool, whereas whey proteins comprise 40% of the total relative protein. These bioactive compounds derived from WPPC have significant potential as innovative ingredients, underscoring the necessity of meticulously isolating and characterizing these components. However, previous research indicates that WPPC production may involve heat-induced complex formations between whey proteins and MFGM. Thus, it is critical to investigate the changes that occur within the WPPC matrix during thermal treatment. In this study, we conducted 2 pilot-scale WPPC in-house trials using Cheddar cheese whey, which included one processed from raw

milk with a single batch pasteurization and another produced from high-temperature, short-time (HTST) milk undergoing a second HTST treatment. Post-production analysis included dynamic light scattering measurements, confocal laser scanning microscopy, Fourier-transform infrared (FTIR) spectroscopy, and reduced and non-reduced SDS-PAGE to examine the insoluble proteins of MFGM dissolved in various reagents. We also quantified β -lactoglobulin through the processing to understand the unfolding and aggregation of β -lactoglobulin. This comprehensive evaluation described how the different treatments impact the interactions between whey proteins and MFGM, along with the formation of insoluble aggregates at the fat surface. By understanding the alterations in whey proteins resulting from these thermal treatments, we aim to enhance the efficiency of isolating valuable compounds such as MFGM proteins, whey proteins, and phospholipids, all of which possess considerable nutritional and economic significance.

Key Words: whey protein phospholipid concentrate (WPPC), protein interaction, milk fat globule membrane (MFGM)

Forages and Pastures Symposium: Building a Resilient Forage Program for Climate Extremes

1240 Dealing with climate change and extreme weather events is part of forage-management future.

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Climate change is evidenced by increasing global mean temperature since industrialization due to retention of incoming solar radiation within the atmosphere due to greenhouse gas accumulation. The upward inflection of temperature has been noted post 1960s. Public awareness has increased due to extreme weather events with dramatic impacts on society including agricultural production. Climatic extremes overlay regional and seasonal climate averages and climate change trends. Hot locations become hotter and moist locations become wetter. Extreme weather events are heat waves, intense precipitation, drought, and tropical cyclones. Compound extremes occur when drought and high temperature occur together, but may include wildfires, unexpected rapid snowmelt, and floods, which are outcomes of the extremes. The frequency and uncertainty of extreme weather occurrence will increase as the mean global temperature continues to increase. Increasing air temperature, the main driver, increases the capacity of air to contain water vapor causing greater evapotranspiration from vegetation or bodies of water. Droughts with high temperature reduce soil moisture during crop-sensitive times. Intense rainfall occurs when water vapor carried by air is released over land masses is problematic at rates greater than can be taken up by soil and crops. Seasonal precipitation may shift to winter and not coincide with periods of crop growth. Intensive water extremes lead to frequent and severe droughts and floods, impacting water availability, infrastructure, and ecosystems services. Adaptation to new normal and uncertainty is crucial to sustainable productivity of the land and related ecosystems. Managing forage, feed crops, and pastures through appropriate regional adaptation to temperature, water use efficiency and improving capacity of soils for rapid infiltration and water storage will be important to reduce risk. Designing herd and infrastructure size to use manure effectively with forage crops to prevent nutrient losses may help reduce harmful outcomes.

1241 Understanding the alternatives and consequences of taking versus not taking action.

L. Silva*, *Animal and Veterinary Sciences Department, Clemson University, Clemson, SC.*

Worldwide, forages are the primary source of feed for livestock production systems due to their diversity of adapted species and lower costs of production. Forage-based livestock operations are complex systems across climates, soil types, genetics, and production systems. Therefore, it is essential to properly implement research-based management practices to support stand production and persistence to maintain a healthy stand against challenges related to extreme weather conditions. Over the past decades, climate trends have shown changes in occurrence and an increase of extreme events (e.g., extended droughts) for the continental US area. These events can lead to issues with production, persistence, and health of forage stands, and overall livestock performance in operations. In recent years, there has been an increasing interest in sustainable production intensification on forage-livestock ecosystems aiming to enhance production, feasibility, resilience, and sustainability while reducing environmental footprint of operations. The

adoption of improved management strategies (e.g., use of mixtures) support the delivery of multiple ecosystem services including carbon sequestration and improved soil fertility and health. Among strategies to be implemented, proper grazing management using adequate harvest frequency and intensity allows for proper plant regrowth and energy reserve recovery which impacts stand health and persistence over time. In addition, the use of rotational stocking improves nutrient cycling and redistribution of excreta throughout the pastures, allowing for improved forage production and nutritive value. Then, the nutrient return associated with proper and constant input of plant litter, dead material and roots through decomposition in perennial forage stands, improves soil nutrient levels and contributes to the organic matter accumulation. Increasing resilience of forage systems is essential and it requires a holistic approach to assess the conditions of each system while considering its specificities, goals and resources.

Key Words: grazing system, livestock production, nutrient cycling, sustainable intensification

1242 The current situation and impacts in animal and human systems.

A. F. Brito*, *University of New Hampshire, Durham, NH.*

Resilience can be broadly defined as the ability to recover from or adjust to misfortunes or changes. Therefore, cropping systems that rely on management strategies to endure and recover from biotic and abiotic stressors such as pests, diseases, droughts, floods, and extreme temperatures can be considered resilient and more equipped to sustainably generate food. The global population is predicted to reach 9.7 billion in 2050 and could peak at 10.4 billion in the mid-2080s, creating pressures on the society and agriculture sector to increase food production to keep up with the growing demand. Specific to the dairy industry, there will be a need to not only increase milk production but also that of milk fat and protein in response to elevated global demand for dairy products. Ideally, production of milk and milk components should increase with reduced competition for land and greenhouse gas emissions intensity, requiring more productive and environmentally friendly forage cropping systems. Further, the dairy industry, and the livestock sector as a whole, need to tackle the “feed-food competition” question, previously defined as “the tensions and tradeoffs between two alternative uses for edible crops: direct consumption by humans versus feeding livestock.” Several levers have been pointed out to potentially solve the feed-food competition question: (1) decreasing total feed demand, (2) reducing food-competing feed demand, (3) increasing food availability, (4) increasing animal feed efficiency, and (5) increasing system efficiency via circular economy. Using cover crops for forage production and soil health, increasing the reliance on forage legumes to improve animal production and farm N self-sufficiency, and feeding digestible forages are some examples that address the feed-food competition issue and promote food security. In fact, dairy and other animal products are excellent sources of EAA and micronutrients, and together contribute to 18% of global calories and 25% of global protein consumption. In summary, building resilient forage cropping systems concurrently with increased production of milk and milk components are key factors to ensure food security and human health.

Key Words: dairy, food security, resilience

1243 Designer forages for the future: Future trends in forage breeding for climate extremes. A. T. Adesogan*, J. C. Dubeux, M. O. Wallau, and E. F. Rios, *University of Florida, Gainesville, FL*.

Increasingly unpredictable extreme weather events due to the changing climate include rising temperatures, sea level rise, prolonged and pronounced droughts, as well as frequent flooding. Forage breeders have a repertoire of classical and cutting edge breeding techniques that are being leveraged to produce more heat, drought, flooding, or salinity-tolerant forages. However, future efforts will need to develop forages that exhibit multidimensional resilience and benefits under various stressors. In addition, future varieties must maintain ecosystem services currently offered by existing varieties despite the change in climate. Traditional forage breeding goals such as increasing forage protein concentration and digestibility as well as pest and disease resistance, should be augmented with more precise objectives such as increasing concentration and bioavailability of limiting amino acids, vitamins or minerals in forage-based diets, reducing antinutritive factors, increasing nutritional stability during storage, improving soil health, inhibiting soil nitrification, stimulating root nodulation and nitrogen fixation, bioremediation capacity, carbon sequestration, and water balance, and increasing the concentration of health-promoting components, such as Mucuna L-dopa, which is used to cure Parkinson's disease, and so on. The ultimate goal should be to develop designer forages that are resilient to multidimensional stressors, more nutritious, easier to digest, less toxic, and more sustainable and health promoting. Achievement of these goals will reduce the need for supplementation with expensive, often imported concentrates, drugs, pesticides and herbicides, and soil amendments, as well as reducing the need to mitigate greenhouse gas emissions from forage-based livestock systems. The power of artificial intelligence to aid hyperspectral remote imaging, data collection and analysis, genetic diversity exploration, and so on should be leveraged to accelerate progress in developing designer forages of the future with multidimensional benefits.

Key Words: designer forage, future forage, forage breeding

1244 Effect of sward type on milk production, intake, and apparent total-tract digestibility of late-lactation dairy cattle. E. Wims^{1,2}, B. McCarthy¹, J. P. Murphy¹, T. F. O'Callaghan², and M. Dineen^{*1}, ¹*Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland*, ²*School of Food and Nutritional Sciences, University College Cork, Co. Cork, Ireland*.

Table 1 (Abstr. 1244). Effect of sward type on milk production, intake, and apparent total-tract digestibility of late-lactation dairy cattle

Item	GO	GC	GCP	SEM	P-value
Milk yield, kg/d	18.2	18.0	17.8	0.87	0.92
Milk crude protein, %	3.88	3.87	3.83	0.09	0.56
Milk fat, %	4.60	4.58	4.23	0.17	0.05
Protein plus fat yield, kg/d	1.53	1.50	1.41	0.06	0.13
Dry matter intake, kg/d	19.7	19.2	19.2	0.55	0.54
Organic matter intake, kg/d	17.4	17.0	16.5	0.47	0.21
Apparent TTDMD, ¹ g/g	0.76 ^a	0.76 ^a	0.69 ^b	0.004	<0.01
Apparent TTOMD, ² g/g	0.81 ^a	0.80 ^a	0.75 ^b	0.004	<0.01

¹TTDMD = total-tract dry matter digestibility.

²TTOMD = total-tract organic matter digestibility.

The objective of this study was to investigate the effect of sward type on milk production, intake, and apparent total-tract digestibility of late-lactation dairy cattle. Nine rumen-cannulated Holstein-Friesian cows, averaging 181 ± 12 DIM and 526 ± 37 kg of BW, were randomly assigned to a treatment sequence in a replicated 3 × 3 Latin square design. The treatments were as follows: perennial ryegrass receiving 50 kg of inorganic N/ha/cut (GO); perennial ryegrass-white clover receiving 25 kg of inorganic N/ha/cut (GC); and perennial ryegrass-white clover-plantain receiving 25 kg of inorganic N/ha/cut (GCP). The pasture feeding rates were adjusted daily to yield refusals of 5% to 10% of intake. In addition, all cows received 0.89 kg of DM of a standard dairy concentrate supplement. The study consisted of three 29-d periods, each period consisting of 21 d of dietary adaptation before an 8-d sample collection period. Data were analyzed using a mixed model (PROC MIXED) that included the fixed effects of treatment and period and the random effect of cow. The GC swards consisted of 83% perennial ryegrass and 17% white clover, whereas the GCP swards contained 38% perennial ryegrass, 7% white clover, 50% plantain, and 5% unsown species. Cows fed GCP tended to have lower milk fat concentration (Table 1). There was no effect of sward type on intake. Cows fed GCP had lower apparent total-tract digestibility (Table 1). The results of this study suggest that high inclusion levels of plantain in pasture swards can negatively affect milk production and total-tract digestibility.

Key Words: sward type, dairy cow, digestibility

Growth and Development 1

1245 Characterization of maternal and fetal vitamin D metabolism during pregnancy in Holstein cows. M. Martin*, A. Fraz, M. Perdomo, and C. D. Nelson, *University of Florida, Department of Animal Sciences, Gainesville, FL.*

Objectives were to test effects of the dose and source of maternal vitamin D on maternal and fetal vitamin D metabolism. Twenty-four lactating Holstein cows between 42 and 96 d gestation at the University of Florida Dairy Research Unit were blocked by day of gestation and randomly assigned to receive cholecalciferol or calcidiol at 0.2 mg or 1 mg in a 2 × 2 factorial arrangement for a total of 4 treatments. Maternal blood was sampled from the coccygeal vein at 0 and 35 d relative to treatment. Cows were slaughtered after 35 d of treatment, and fetal blood and fetal and maternal renal samples were collected for analysis of vitamin D metabolism. Data were analyzed using mixed models with fixed effect of treatment and random effect of block. Plasma concentrations of major vitamin D metabolites are shown in Table 1. Calcidiol was more effective than cholecalciferol at altering fetal and maternal vitamin D metabolism. Relative abundance of mRNA transcripts for *CYP27B1*, encoding the 25-hydroxyvitamin D 1 α -hydroxylase in dam kidney samples, did not differ among treatments. An interaction between dose and source was observed for *CYP27B1* in fetal kidney samples with *CYP27B1* lowest in fetuses from dams fed 1 mg calcidiol compared with other treatments. Maternal calcidiol supplementation was more effective than cholecalciferol in altering maternal and fetal vitamin D metabolism. Dose-source interaction affected fetal renal *CYP27B1* expression, suggesting an influence of maternal vitamin D on fetal metabolism.

Key Words: vitamin D, gestation, Holstein

1246 The impact of preweaning plane of nutrition and heat abatement on mammary gland development of dairy calves. M. C. Guenther*, A. Amato², M. Sfulcini³, G. A. Larsen¹, C. G. Savegnago⁴, A. J. Geiger⁵, S. Tao⁴, and J. Laporta¹, ¹*University of Wisconsin-Madison, Madison, WI*, ²*Università degli Studi di Messina, Messina, Italy*, ³*Università Cattolica del Sacro Cuore, Piacenza, Italy*, ⁴*University of Georgia, Athens, GA*, ⁵*Zinpro Corporation, Eden Prairie, MN*.

Heat stress (HS) during the preweaning period alters calf growth, while enhanced nutrition (EN) improves mammary gland (MG) development. Yet, the potential interaction between these factors in early life is unknown. This study aimed to evaluate MG development in dairy heifers fed differing planes of nutrition and exposed to environmental

HS or provided heat abatement (HA, applied using positive pressure tube ventilation system) during the preweaning period in a subtropical summer (average THI = 78.4). Holstein heifers at 2 d of age (n = 64) were assigned to a 2 × 2 factorial arrangement of treatments consisting of 2 planes of nutrition (CON; 4 L/d or EN; 9 L/d; CP 26%, fat 20%) with or without HA (HA or HS; airspeeds: 1.5 vs. 0.3 m/s, respectively). Calves were individually housed outdoors under a shade structure with ad libitum access to starter grain. The HA heifers had decreased rectal temperatures and respiratory rates compared with HS. External MG measurements (teat length, distances between teats) were assessed using digital calipers, and MG ultrasonography was performed to visualize and quantify parenchyma (mPAR) and fat pad (mFP) areas at 28, 42, and 56 d of age. A subset of heifers (n = 24) were euthanized at 62 ± 0.2 d to harvest the udder. The mPAR was dissected away from mFP and weighed. Data were analyzed using PROC MIXED in SAS. The EN and HS heifers had longer front and rear teat lengths (main effects, $P \leq 0.02$). The estimated gland area, calculated using distances between teats, was larger for EN and HA heifers (main effects, $P \leq 0.05$). Measured by ultrasonography, HA did not affect mFP or mPAR size; however, there was an interaction between nutrition and day, where mFP and mPAR sizes were similar at 28 d but larger in EN heifers at 42 and 56 d of age ($P < 0.01$). At euthanasia, EN and HS heifers had greater ($P < 0.01$) MG and mFP weights. The HA did not affect mPAR weight ($P = 0.95$); however, EN heifers ($P < 0.01$) had greater mPAR weights. Results indicate that the MG in early life is experiencing developmental plasticity and can be impacted by EN nutrition and HA, but these factors do not appear to act synergistically.

Key Words: thermoregulation, ventilation, perinatal programming

1247 Third-generation impacts of heat stress on mammary gland growth and development. G. A. Larsen*, M. Reimann, E. Cowan, and J. Laporta, *University of Wisconsin-Madison, Madison, WI*.

Heat stress exposure of F_0 cows during the dry (late-gestation) period reduces their milk potential, the F_1 and F_2 early-life mammary parenchyma (mPAR) and fat pad (mFP) size, and milk yield at maturity. While the F_1 generation exhibits reduced body growth, the F_2 grows at similar rates to their cooled counterparts. Herein, we investigated the impact of maternal late-gestation heat stress (HT) on body growth and mammary development of their great-granddaughters (F_3). Eighty-two F_0 multipa-

Table 1 (Abstr. 1245). Effect of maternal vitamin D dose and source on plasma vitamin D metabolites

Metabolite ¹	Cholecalciferol		Calcidiol		SE	P-value		
	0.2 mg	1 mg	0.2 mg	1 mg		Dose (D)	Source (S)	D × S
Maternal								
25(OH)D ₃ , ng/mL	39.8	43.0	38.4	76.5	3.4	<0.01	<0.01	<0.01
24,25(OH) ₂ D ₃ , ng/mL	1.7	2.5	1.6	3.1	0.5	0.05	0.58	0.55
1,25(OH) ₂ D ₃ , pg/mL	23.6	20.1	20.0	24.2	1.7	0.84	0.86	0.04
Fetal								
25(OH)D ₃ , ng/mL	7.9	5.8	5.9	11.4	0.85	<0.01	0.04	<0.01
24,25(OH) ₂ D ₃ , ng/mL	1.7	1.3	1.2	1.7	0.22	<0.01	0.97	0.03
1,25(OH) ₂ D ₃ , pg/mL	50.2	48.9	46.0	38.9	6.65	0.09	0.25	0.63

¹25(OH)D₃ = 25-hydroxyvitamin D₃, 24,25(OH)₂D₃ = 24,25-dihydroxyvitamin D₃, and 1,25(OH)₂D₃ = 1,25-dihydroxyvitamin D₃.

rous pregnant Holstein cows were dried in a subtropical summer, with access to heat abatement (freestall barn, fans, and water soakers for cooling, CL) or not (barn and natural ventilation, HT) for the last 54 d of gestation. The F_1 daughters born to F_0 were in-utero HT (HT_{F1} , $n = 36$) or CL (CL_{F1} , $n = 37$) and raised as a cohort; F_2 heifers born to HT_{F1} (HT_{F2} , $n = 12$) and CL_{F1} (CL_{F2} , $n = 17$) were raised identically and gave birth to HT_{F3} ($n = 9$) and CL_{F3} ($n = 10$) females. The F_3 heifers were raised until d 70. Body weight, hip height, wither height, and chest girth were measured on d 1, 28, 56, and 70. Mammary gland teat length (TL) and the left–right and front–rear teat distances (TD) were measured at d 28, 56, and 70. At d 28 and 70, mammary ultrasounds were performed to quantify mPAR and mFP areas. On d 70, a biopsy was taken ($n = 6$ /group) to quantify luminal (L) and non-luminal (NL) epithelial areas and proliferating cells (Ki67+) in the epithelial (MEC) and stromal cell compartments. Data were analyzed using PROC MIXED and PROC GLIMMIX in SAS. Heifer growth trajectory (birth–70 d) was similar between CL_{F3} and HT_{F3} (all, $P > 0.3$). The TD and TL, and total mPAR and mFP areas, were similar between groups (all, $P > 0.2$). At d 70, CL_{F3} tended to have fewer NL epithelial structures than HT_{F3} but higher L epithelial area (3.3 vs. $4.5 \mu m^2$; 14.2 and $10.8 \mu m^2$, respectively, $P < 0.09$). The proportion of Ki67+ cells was greater overall and in the MEC compartment of CL_{F3} heifers than HT_{F3} (38 vs. 23% , $P < 0.01$). These effects can be considered transgenerational as the F_0 HT had no direct contact with the F_3 generation, emphasizing the importance of heat abatement at all life stages.

Key Words: transgenerational, heat stress, mammary

1248 Effect of in utero heat stress and rumen-protected choline on Holstein heifer development. M. A. T. de Bari^{*1}, K. Estes², C. Zimmerman², H. Olmo¹, D. Onan-Martinez¹, A. Arteaga¹, J. Lance¹, and G. E. Dahl¹, ¹University of Florida, Gainesville, FL, ²Balchem Corporation, Montvale, NJ.

Dry period heat stress (HT) negatively impacts fetal development, leading to life-long consequences on organ growth, survival and productivity. While *in utero* factors influence development, the role of altered nutrient delivery, particularly additional methyl donors, remains unclear. This study investigated the effects of late-gestation HT and the potential for choline supplementation to mitigate these effects. We hypothesized that HT in late gestation will alter patterns of gene expression and methylation patterns, but supplementing the dam with rumen-protected choline (RPC) could result in methylation profiles resembling those calves from cooled dams. Late-gestation Holstein cows ($n = 69$) in a freestall barn were assigned to HT (HT , $n = 18$), HT with RPC (HTC, $n = 15$), cooling (CL, $n = 18$) or CL with RPC (CLC, $n = 19$). A cooling system with shade, fans and soakers was provided to CL and CLC cows, whereas HT and HTC cows had only shade. RPC (30 g/d) was mixed with TMR. Female calves ($HT = 7$, $HTC = 8$, $CL = 6$, $CLC = 9$) were weighed and had blood samples collected at birth. They were raised under shade and fans, fed grains and milk replacer, and slaughtered at 60 (± 13) d for organ collection. Statistical analyses were performed using the PROC MIXED procedure of SAS in a 2×2 factorial arrangement, considering main effects and interactions, significance was set at $P < 0.05$. Gestation length and dry period were shorter in HT ($HT+HTC$) versus CL ($CL+CLC$) (272 ± 1.1 vs. 275.5 ± 1 d, $P = 0.02$; 42.6 ± 1.4 d vs. 46.6 ± 1.3 d, $P = 0.03$). Colostrum yield did not differ between HT and CL groups ($HT+HTC$; 3.8 ± 0.7 vs. $CL+CLC$ 4.5 ± 0.6 kg, $P = 0.4$). The HT calves tended to be born lighter than CL calves (34.4 ± 1.4 vs. 38.0 ± 1.4 kg; $P = 0.08$). The HT calves had smaller ovaries than CL ($HT+HTC$ 3.2 ± 0.4 vs. $CL+CLC$ 4.2 ± 0.4 , $P = 0.03$). Thymus weight tended to be lighter in HT compared with CL calves ($HT+HTC$; 0.40

± 0.03 vs. $CL+CLC$; 0.47 ± 0.03 g ($P = 0.07$). Adrenal gland weight tended to be heavier in HT calves relative to CL ($HT+HTC$; 6.03 ± 0.05 vs. $CL+CLC$ 4.7 ± 0.5 g; $P = 0.06$). These results suggest that RPC supplementation does not mitigate the negative effects of HT in calves at the organ level. However, the potential for epigenetic modifications and metabolic adaptations requires further study.

Key Words: epigenetics, methyl donors, dry period

1249 Preliminary genetic parameters for metabolizable energy intake, metabolic body weight, and average daily gain in Holstein calves. A. G. R. Phillips^{*1}, B. O. Mankuola¹, F. Miglior^{1,2}, F. S. Schenkel¹, C. F. Baes¹, and R. E. Jahnel¹, ¹Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada, ²Lactanet, Guelph, ON, Canada.

This abstract is part of the ADSA-Graduate Student Competition: Production (MS Oral Semifinalists).

Early-life feed intake and growth critically impact calf welfare and long-term performance. Genetic selection for feed intake in dairy calves could improve herd efficiency by breeding animals that meet growth requirements with minimal feed resources, lowering rearing costs and environmental impact. Furthering industry understanding of calf feed efficiency would support investigating whether there is a correlation with feed efficiency in adult animals, ultimately improving lifetime efficiency in dairy cattle. The metabolizable energy intake (MEI) derived from milk replacer and calf starter could serve as a measure of calf feed efficiency. This study aimed to investigate MEI and its potential use as a tool to select feed-efficient dairy calves. Using 44,512 daily feed intake records from 908 Holstein dairy calves raised at the Ontario Dairy Research Centre, 2 different statistical models were employed. A univariate random regression model was used to estimate genetic parameters of daily MEI and examine how its genetic variance fluctuates over time. Average heritability and repeatability for daily MEI were 0.16 ± 0.03 and 0.45 ± 0.02 (average SE of estimates), respectively. Genetic correlations between daily MEI estimates ranged between -0.39 and 0.99 , likely reflecting calf gastrointestinal maturation. Second, a multiple-trait repeated-records model for MEI, metabolic body weight (MBW), and average daily gain (ADG) in the pre- and postweaning periods were fit. Heritability for MEI, MBW, and ADG were 0.33 ± 0.05 , 0.54 ± 0.06 , and 0.20 ± 0.04 , respectively, for preweaning period, and 0.38 ± 0.02 , 0.51 ± 0.07 , and 0.20 ± 0.04 for postweaning period. Positive genetic correlations were estimated between MEI and MBW (0.72 ± 0.06), MEI and ADG (0.86 ± 0.06), and MBW and ADG (0.76 ± 0.06), for preweaning period, whereas correlations were lower in postweaning period (0.44 ± 0.11 , 0.45 ± 0.11 , and 0.72 ± 0.07 , respectively). Study results improve understanding of the relationships between potential calf feed efficiency related traits in the preweaning and postweaning periods.

Key Words: calf growth, feed efficiency, genetics

1250 Blood epigenetic signatures of early-life nutrition in dairy calves: Exploring the implications on immune system. G. Costa Monteiro Moreira^{*1,2}, A. Chaulot-Talmon^{1,2}, F. Ali^{1,2}, C. Staub³, P. Salvetti⁴, J. Dupont⁵, H. Kiefer^{1,2}, and H. Jammes^{1,2}, ¹BREED, Université Paris Saclay, UVSQ, INRAE, Domaine de Vilvert, Jouy-en-Josas, France, ²Ecole Nationale Vétérinaire d'Alfort, BREED, Maisons-Alfort, France, ³INRAE Centre Val de Loire, Physiologie Animale de l'Orfrasière, Nouzilly, France, ⁴ELIANCE Station de phénotypage, Le Perroi, Nouzilly, France, ⁵CNRS, IFCE, INRAE, Université de Tours,

The early weeks of life are critical for a young ruminant's future productivity and performance. Nutritional intake must be carefully balanced, as excessive feeding can negatively impact other physiological functions. Here, we aim at identifying blood epigenetic biomarkers reflecting the effects of different feeding strategies on cow's health across 2 generations, providing insights into intergenerational effects and their persistence throughout life. Blood samples were collected from Holstein calves/heifers from both first and second generations (G1 and G2) at 1, 6, 9, 12, and 18 mo of age, either under an optimized feeding (OPT) management (ADG of 1,000 g/d) or fed with a conventional (CTL) diet (ADG of 800 g/d), with a minimum of 10 individuals from each group/time point. Blood methylomes were profiled using bisulfite sequencing/RRBS. Differential methylation analysis was performed using *methylKit* (logistic regression method; $\Delta\text{meth} \geq 10\%$, adjusted P -value < 0.05). Preliminary analyses compared OPT vs. CTL (i) at 6 mo of age in G1, (ii) at 9 mo of age in G1, and (iii) at 6 mo of age in G2, identifying 1,212, 1,749, and 964 differentially methylated cytosines (DMCs), respectively. Notably, 333 DMCs were shared between comparisons (i) and (ii), suggesting persistence of epigenetic marks within the same generation. Additionally, 169 DMCs were common to comparisons (i) and (iii), suggesting intergenerational effects and/or a systematic response to feeding strategies. Moreover, 77 DMCs were consistently detected across all comparisons, suggesting both persistence and intergenerational effects and among them, a hypermethylated DMC co-localized with the *F2RL1* gene, a key regulator of inflammatory responses and immune function. Also, a hypomethylated DMC co-localized with the *OSBPL3* gene, involved in lipid metabolism/fat accumulation, aligning with dietary differences between groups. Taken together, our findings provide novel insights into the epigenetic regulation of immune and metabolic pathways in response to early-life nutrition, shedding light on both short-term and intergenerational effects of feeding strategies in dairy cattle.

Key Words: nutrition, epigenetics, immune system

1251 Associations of preweaning lung consolidation on the periparturient peripheral leukocyte transcriptome in primiparous Holstein dairy cows. L. C. Richmond*, J. A. Velasco, S. P. Ficklin, C. T. Cauchy, L. M. Parrish, K. N. Heaton, C. C. Figueiredo, and C. S. McConnel, *Washington State University, Pullman, WA.*

The periparturient period in dairy cattle demarcates a critical period of health due to substantial physiological demands (e.g., metabolic, immunologic) related to parturition and lactation. The ability to successfully adapt to these changes ultimately dictates their productive life. This study aimed to explore the peripheral leukocyte transcriptome for potential intrinsic immune factors driving the periparturient period relative to evidence of previously diagnosed calfhood disease (e.g., bovine respiratory disease [BRD]). Calves enrolled within the first 10 d of life (May 2021: $n = 61$; May 2022: $n = 60$) were evaluated weekly for 12 wk via thoracic ultrasonography. Cows with a successful first lactation transition ($n = 25$) were eligible for further analyses based on retrospective classification of either "healthy" (no consolidation; $n = 10$) or "chronic" (≥ 1 lung lobe consolidated for ≥ 3 wk in duration; $n = 15$). Leukocytes were isolated from whole blood samples collected during the first and second week of lactation via modified Ficoll-Paque separation followed by RNA extraction (miRNeasy kit) and sequencing. The RNA sequence analysis conducted with EdgeR identified 1,370 upregulated and 786 downregulated differentially expressed genes (DEGs). The

putative functions of DEGs were obtained with functional enrichment analysis via FUNC-E v2.0.1. Cows with preweaning BRD had notable immune regulation differences (e.g., downregulated gene clusters ($n = 9$) associated with cell migration and natural killer cell regulation; upregulated gene clusters ($n = 27$) related to stress response signaling and proinflammatory cytokine regulation) when compared with cows with no preweaning BRD. These immunoregulatory differences suggest that preweaning disease may have underlying and persistent impacts on immune factors driving the periparturient period. Further exploration of these factors may provide a complementary insight into associated disease phenotypes and transition cow management. Additionally, this can further inform genetic selection strategies aimed at reducing production loss while increasing disease resiliency.

Key Words: bovine respiratory disease (BRD), periparturient, inflammation

1252 Long-term effect of prebiotic, probiotic, and synbiotic additives fed during the preweaning period on subsequent milk production of Holstein dairy cows. M. M. Muhffel*, S. S. Aly¹, P. M. Lucey³, I. J. Lean², and H. A. Rossow¹, ¹*Veterinary Medicine Teaching and Research Center, University of California–Davis, Davis, CA*, ²*Scibus, Camden, NSW, Australia*, ³*Elanco Animal Health, Greenfield, IN.*

Prebiotics and probiotics have emerged as feed additives that enhance the health and productivity of dairy cows. They benefit the host by modulating the gut microbiome playing a crucial role in digestion, immunity, and overall animal health. The study's objective is to investigate the long-term impact of feeding preweaning Holstein calves prebiotic, probiotic, or synbiotic additives on their future milk production. This study is a retrospective analysis of milk production records from dairy cows that were randomized at birth to one of 4 daily treatments administered during the preweaning period: (1) prebiotic (PRE; 7 mL of *Saccharomyces cerevisiae* yeast culture), (2) probiotic (PRO; *Bacillus subtilis* and *Lactobacillus plantarum*, delivering approximately 1 billion and 250 million cfu per head per day, respectively), (3) Treatments 1 and 2 (SYN; synbiotic), and (4) no additive (CON; control). The study was conducted on a dairy in Fresno, California, involving 1,296 Holstein cows contributing to 2,920 lactations distributed over their first 3 lactations. Monthly test-day records for milk yield, fat, and protein were used to calculate the energy-corrected milk (ECM), standardized to 4% fat and 3.3% protein, totaling 26,975 monthly test-day milk records. A 2-piece splines mixed-effect regression model evaluated the effect of treatments on ECM yield. For the first lactation, ECM yield was estimated at 28.66 kg on the first DIM, peaked at 42.1 kg, and declined to 21.34 kg by 305 DIM. For lactation ≥ 2 , ECM yield was 41.06 kg at 1 DIM, 54.2 kg at peak, and 33.74 kg at 305 DIM. The SYN had increased ECM yield by 1.00 kg/d compared with CON ($P = 0.04$, 95% CI: 0.02–1.99 kg/d). No differences in ECM yield between PRE or PRO and CON were observed. These findings suggest that supplementing SYN in preweaning calves' milk increases milk production across the first 3 lactations of their lifetime.

Key Words: dairy, synbiotic, energy-corrected milk (ECM)

1253 Short- and long-distance transportation: Survival and growth of dairy and dairy-beef cross calves. G. M. Schuenemann*,¹ and J. M. Pineiro², ¹*Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH*, ²*Department of Animal Science, Texas A&M University, College Station, TX.*

The objective of this retrospective observational study was to assess the relationship of transportation duration (0.5, 8, 17, or 24 h) with calf survival and preweaning average daily gain (ADG). A total of 392,064 calves (dairy [D] = 125,901 and dairy-beef cross [DB] = 266,111) born from HxJ dams from 15 dairies under the same overall management were assessed from January 2022 to March 2024. Calves (male = 125,953 and female = 146,163) were fed 3.8 L of colostrum within 2 h of birth and the conditioning protocol before transportation consisted of 4 consecutive feedings (each of 2 L per calf, the fourth milk feeding was mixed with 1.9 L electrolyte). Calves that drank 4 consecutive meals after colostrum administration and without experiencing signs of sickness were considered fit to transport. Gestation length (GL), parity, dystocia, failure of passive transfer (FPT), calf birth weight, birth season, calf age at transportation, calf sex, calf breed, and birth year were recorded. Calf diseases (diarrhea and pneumonia) and mortality upon arrival at calf-raising facility (CM) and up to weaning (CMW; 60 ± 3 d) were recorded. Data were analyzed using GLIMMIX or MIXED procedures of SAS. Parity, calf health, calf birth season and year, FPT, dystocia, calf sex, calf breed, and calf age at transportation were associated with calf survival ($P < 0.05$). Overall CM was 0.0148%, and it did not differ by transportation duration ($P = 0.13$). Overall CMW was 2.49% but varied: 3.56% (0.5 h), 1.01% (8 h), 2.18% (17 h), and 1.55% (24 h; $P < 0.05$). Increased ($P < 0.05$) CMW were associated with FPT, calf diseases, female D calves born to heifers and birth season (summer), and GL. The ADG (kg/d) did not differ for DB calves (8 h = 0.86 ± 0.04 or 17 h = 0.82 ± 0.02), regardless of parity and transportation duration. The D calves transported 24 h (0.76 ± 0.01) had higher ADG (kg/d) compared with those D calves transported 0.5 h (0.65 ± 0.02 ; $P < 0.05$), primarily due to the confounding effect of parity. While a causal relationship between transportation duration and survival or ADG cannot be established, extended transportation duration did not negatively affect survival or ADG.

Key Words: calf, transportation duration, survival and growth

1254 Impacts of plane of nutrition and heat abatement during the preweaning period on growth of dairy heifers. C. G. Savagnano^{*1}, A. M. Roper¹, Y. B. Amancio¹, J. Gao¹, T. N. Marins¹, N. Andriotti¹, M. C. Guenther², A. Amato^{2,3}, J. Laporta², A. J. Geiger⁴, and S. Tao¹, ¹Department of Animal and Dairy Science, University

of Georgia, Athens, GA, ²Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI, ³Department of Veterinary Sciences, Università degli Studi di Messina, Messina, Italy, ⁴Zinpro Corporation, Eden Prairie, MN.

Calf performance is influenced by nutrition and environment. We aimed to examine the impact of milk replacer (MR) feeding rates and heat abatement on growth performance of Holstein heifers during summer. From 2 to 4 d of age (DOA, $n = 167$), heifers were assigned to 4 treatments based on BW (36.8 kg) in a 2×2 factorial arrangement of 2 feeding rates: enhanced (ENH, 9 L/d of a 26% CP and 20% fat MR, 12.5% solid) or control (CON, 4 L/d), and 2 environments: cooling (CL, via positive pressure tube ventilation achieving 1.5 m/s wind speed, and day-time misters) or no-cooling (NC). Calves were housed outdoors in individual wire hutches under shade until 70 DOA. Calves were fed MR at 0500, 1300, and 2200 h until 42 DOA. The CON group was weaned by feeding $1 \times$ (0500 h, 2 L) from 43 to 56 DOA, whereas ENH heifers underwent a step-down approach (43–49 DOA: $2 \times$, 0500 and 1300 h, 6 L; 50–56 DOA: $1 \times$, 0500 h, 3 L). Calf starter (CS) was provided *ad libitum* and intake recorded from 28 to 70 DOA. From 71 to 180 DOA, all heifers were grouped-housed on pasture and managed identically. Body weight was measured at 2–4, 28, 42, 56, 70, 110, and 180 DOA. Respiration rate (RR) and rectal temperature (RT) were recorded $3 \times$ a week (1600 h) until 70 DOA ($n = 16/\text{treatment}$). Data were analyzed by PROC MIXED procedure of SAS. Maximum, minimum, and average air temperature and relative humidity of the hutch area were 33.3, 23.7, 27.6°C and 87.7, 57.8, 76.2%, respectively. Relative to NC, CL lowered RR and RT, and ENH increased RT than CON (main effects, $P < 0.01$). The ENH treatment led to a greater ($P < 0.01$) MR intake, but CL did not affect MR intake. From 28 to 56 DOA, ENH lowered ($P < 0.01$) CS intakes than CON (nutrition $\times$ time, $P < 0.01$), and CL tended ($P = 0.07$) to increase CS intake from 57 to 70 DOA than NC (environment $\times$ time, $P = 0.08$). Until 180 DOA, ENH resulted in greater ($P < 0.01$) BW and ADG than CON, whereas no ($P \geq 0.20$) cooling effect was observed. Providing a higher plane of MR nutrition improves the growth performance of heifers during the pre- and postweaning periods. Cooling effectively lowered RT and RR and increased CS intake, yet no synergistic effects were observed with the plane of nutrition.

Key Words: cooling, nutrition, calf growth

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1259 Review and revision of the St-Pierre dairy cow model to estimate losses from heat stress. M. Malekkhahi¹, I. Flamenbaum², and A. De Vries^{*1}, ¹University of Florida, Gainesville, FL, ²Cowcooling Flamenbaum and Seddon, Jales, SP, Brazil.

In 2003, St-Pierre et al. (STP) published equations that estimated the economic losses from heat stress in major livestock industries in the USA, including dairy cattle. Our objective was to review and revise the dairy cow equations in STP that predict how milk yield, dry matter intake, days open, reproductive culling, and death risk are affected by heat stress as measured by functions of the temperature-humidity index (THI) such as THI-load and THI-maximum. First, the published equations for changes in days open and reproductive culling clearly contain errors. We used the published results in STP to derive new equations to replace the erroneous equations. We found that a second-degree polynomial fitted the association between the increase in reproductive culling and THI-load well. A simple linear equation fitted the association between days open loss and THI-load. However, we found the relationship between THI-load and days open in STP to be overestimated by a factor of 2. Second, we fitted new equations for milk loss and dry matter intake loss based on more recent literature (30 studies published between 1990 and 2023). The new equations predict lower milk losses and lower dry matter intake losses at higher THI-maximum than the equations in STP. This suggests that losses at higher THI may be overestimated in STP. Third, we developed equations for reductions in milk fat and milk protein as functions of THI. Based on a literature review, we assumed that under a high heat stress load such as during summer conditions in the Florida, both fat and protein percentages would decrease by 0.20%-points. Fourth, we did not find sufficient evidence to update of the equations for reproductive culling and death risk as published in STP. Collectively, we concluded that several equations in STP overestimated losses due to heat stress as measured by THI. However, current THI-thresholds at which heat stress losses start are lower than assumed in STP and average THI have increased in the last 2 decades. Further analyses will estimate the profitability of mechanical cooling with the revised equations.

Key Words: climate, heat stress, cooling

1260 Milk spectra analysis as a tool for predicting heat stress in dairy cows. M. Bahadi¹, A. Ruiz-Gonzalez², L. Fadul-Pacheco¹, and D. E. Rico^{*3}, ¹Lactanet, Dairy Center of Expertise, Sainte-Anne-de-Bellevue, QC, Canada, ²Université Laval, Québec, QC, Canada, ³CRSAD, Deschambault, QC, Canada.

Fourier-transform infrared (FTIR) spectroscopy of milk samples presents a promising approach for identifying heat stress risk in dairy cows. Milk spectra from 4 independent heat stress induction experiments were used to identify spectral features with predictive potential. Each experiment followed a Latin square design, providing 12 observations per treatment and sampling time. Treatments were designed to isolate the effects of heat stress at a consistent feed intake level: (1) heat stress (HS; cyclical THI variation between 72 and 82) and (2) pair feeding under thermoneutrality (PF; THI = 64). Milk samples were collected at baseline (d 0) and at subsequent time points (d 3, 7, 10, and 14 of each period). Spectral regions 3,061–2,803 cm⁻¹, 1,797–1,681 cm⁻¹, and 1,612–925 cm⁻¹ were analyzed. Principal component (PC) analysis combined with mixed modeling identified significant treatment effects ($P < 0.001$) in PC2, PC4, and PC5. Key spectral features in loading

spectra of these PCs correspond to protein, unsaturated fatty acids, and energy-related metabolites, such as b-hydroxybutyrate, acetone, and citrate. These findings suggest that these metabolites may serve as discriminative indicators of heat stress. To further differentiate HS cows from baseline, a partial least squares discriminant analysis (PLS-DA) model was developed, excluding spectra from d 2 and 3 and all PF treatment spectra. The model achieved a misclassification rate of 7%, with a Matthew's correlation coefficient of 0.852, sensitivity of 0.95, and specificity of 0.93. Spectral features contributing to group differentiation included fat and urea, in addition to previously identified metabolites. Although heat stress-induced changes in milk spectra effectively distinguished treatment groups, the metabolic impact of feed restriction in PF cows must be considered, as ad libitum-fed animals may provide a more appropriate reference. These findings suggest that milk spectral analysis may serve as a valuable tool for detecting metabolic stress due to hyperthermia in dairy cows.

Key Words: milk spectroscopy, heat stress, metabolic biomarker

1261 Commercial application of “smart” technologies to reduce water usage when cooling dairy cows. H. Olmo*, M. A. T. de Bari, D. Onan-Martinez, J. Magalhaes, M. Siregar, J. Lance, I. M. Toledo, and G. E. Dahl, University of Florida, Gainesville, FL.

Optimal performance of dairy cows during all phases of the lactation cycle, is crucial to mitigate the effects of heat and humidity, as these factors can negatively impact animal health and productivity. To alleviate the negative effects of heat stress, shade, ventilation and active cooling systems are widely used strategies. The objective of this study is to evaluate the effectiveness of an automated “smart” system (AgPro, Paris, TX) for control of soaker output, compared with a conventional approach that relies on set timing after reaching a specified temperature threshold. We hypothesize that the “smart” system will reduce water use but be as effective as the conventional system in maintaining body temperature and respiration rate and not impact milk yield. Under commercial settings, we evaluated the water usage (soakers and water intake of cows) in 4 pens of lactating cows ($n = \sim 460$ cows/pen); 2 pens with smart soakers (LSS) and 2 pens with the conventional system (LC), and 4 pens of dry cows ($n = \sim 170$ cows/pen); 2 pens with smart soakers (DSS) and 2 pens with the conventional system (DC). Respiration rate (RR; bpm), rectal temperature (RT; °C), and skin temperature (ST; °C) were measured thrice weekly in a random group of 30 cows in each pen. Milk production (MP; kg/d), dry matter intake (DMI; kg/d), water intake (WI; L/d), and soaker system water usage (WU; L/d) per pen were recorded daily. Preliminary results indicate no differences in both lactating and dry cows for RT, RR, ST, and MP. A lower WI (3.9 vs. 3.6 ± 0.2 L/pen/d $\times 1,000$; $P = 0.05$) and higher DMI (12.8 vs. 13.6 ± 0.3 kg/cow/d; $P = 0.009$) was observed in the DSS pens. Water usage was significantly higher in both LC and DC compared with LSS and DSS (43.3 vs. 25.5 ± 0.8 , 22.1 vs. 12.4 ± 0.8 L/d $\times 1,000$; $P < 0.001$). These results suggest that the automated “smart” system is as effective as a conventional soaker system to actively cool cows while using about half the amount of water.

Key Words: dairy cattle, cooling system, water use

1262 Real-time milk yield prediction and health monitoring system for dairy cows. M. J. Gote*, D. Meuwissen¹, L. D'Anvers¹,

I. Adriaens^{1,2}, and B. Aernouts¹, ¹KU Leuven, Geel, Belgium, ²Ghent University, Gent, Belgium.

The widespread use of automatic milking systems has increased the availability of high-frequency sensor data, especially in Western Europe. This increases reliance on automated disease detection for more targeted farmer-herd interactions. In this study, we implemented a linear mixed model predicting quarter-level milk yield, a moving range control chart identifying deviations, and alert rules for semi-real-time detection. The objective was to give a proof of concept for the technical feasibility of the setup and to specify further milk-production-related parameters to identify disease-related alerts. Dataset 1 comprises a 3-mo trial on 2 farms with 225 unique lactations, generating 175 farmer-verified alerts. Dataset 2 includes data from 10 mo for one farm with 106 unique lactations yielding 586 alerts, which were compared with a treatment register. Alerts were classified as clinical, subclinical, dry-off, or unspecified fluctuations (DS1: 24, 20, 43, 88; DS2: 72, 105, 87, 322). Significance was tested using a Kruskal-Wallis test with Dunn's pairwise comparisons. Comparing clinical to unspecified alerts, key distinguishing features of milk yield deviations included total case length (4 vs. 1 d, $P < 0.001$), mean milking interval (8.5 vs. 7.8 h, $P < 0.01$), and total relative loss (−17.3% vs. −7.0%, $P < 0.001$) on d −1 to +3 from the alert. Based on the second dataset, we identified features for differentiation of mastitis, ketosis, and unspecified. These were case length (5 vs. 3 vs. 1 d, $p_{m-u} < 0.001$), days in milk at detection (166 vs. 25 vs. 123 d, $p_{m-u} < 0.001$, $p_{k-u} < 0.001$, $p_{m-k} < 0.001$), mean milking interval (8.9 vs. 7.8 vs. 7.8 h, $p_{m-k} < 0.001$, $p_{m-u} < 0.001$), and the relative loss difference between quarters (−20.5 vs. 0, −10.8 vs. −5.8, −7 vs. 0.1%, $p_m < 0.001$, $p_k < 0.05$, $p_u < 0.001$). Results confirm the technical possibility of semi-real-time milk yield prediction for disease detection, but improvements are needed for daily decision-making. Further research is required to utilize the found difference in milk loss dynamics to reduce non-disease alerts and integrate additional sensor data, such as electrical conductivity and activity, to build a more holistic disease detection model.

Key Words: precision livestock farming, automatic milking system, milk yield prediction, disease detection

1263 Assessing strategies for deep learning-based body condition score prediction across Holstein and Jersey cattle using computer vision techniques. T. Abdulrahman^{*1}, C. Saha¹, B. B. Medeiros¹, J. Hooker¹, and A. A. C. Alves^{1,2}, ¹Department of Animal and Dairy Science, University of Georgia, Athens, GA, ²Institute for Integrated Precision Agriculture, Athens, GA.

Body condition score (BCS) is a critical indicator of dairy cattle health, welfare, and productivity. However, traditional BCS methods rely on subjective, labor-intensive visual inspections. This study investigated strategies for automated BCS prediction in Holstein (H) and Jersey (J) cattle based on computer vision systems. A total of 8,155 images (4,340 for H and 3,815 for J) were collected using an Intel RealSense D435 camera installed at milking parlors and paired with RFID information to retrieve animal IDs. The collected RGB and depth images were segmented using the U-Net architecture. The BCS was measured weekly by 2 trained observers on a 1-to-5 scale. A ResNet50-based model with focal loss was implemented to handle class imbalance and hard-to-train examples. The model's accuracy was assessed based on a restricted 5-fold cross-validation, ensuring that all data from the same cow were assigned exclusively to either the training or validation folds. Different strategies were tested based on the image type and training population. These included training on both Holstein and Jersey breeds while validating separately (HJ_Depth, HJ_RGB), as well as using

data from each breed individually for training and validation (J_Depth, J_RGB, H_Depth, H_RGB). For Holstein, HJ_Depth achieved the highest accuracy (92.4%) at a 0.5-unit deviation, surpassing H_Depth (91.2%), H_RGB (83.4%), and HJ_RGB (85.6%). At a 0.25-unit deviation, accuracy ranged from 61.2% to 71.6%, with HJ_Depth performing best. For Jersey, HJ_RGB and HJ_Depth achieved similar performance at a 0.5-unit deviation (89.2% × 88.8%), outperforming J_Depth (88.7%) and J_RGB (86.4%) strategies. At a 0.25-unit deviation, accuracy ranged from 71.6% to 72.6%. Exact prediction accuracy (no deviation) ranged from 40% to 46.9%. Our findings highlight that combining H and J breeds in training can benefit BCS prediction for both breeds. In general, depth images provided the best performance for Holsteins, whereas for Jersey, using either RGB or depth provided similar performance on the combined data.

Key Words: dairy cattle breed, deep learning, depth image

1264 Surplus springers or crossbred beef? Net present value of 2 strategies for non-replacement animals. J. S. Adamchick^{*1}, K. R. Briggs², and D. V. Nydam¹, ¹Cornell University College of Veterinary Medicine, Ithaca, NY, ²Fairlife, Chicago, IL.

We compared the discounted net cash flow (NPV) of 2 strategies for investing in non-replacement (surplus) animals for a typical US dairy herd. We evaluated: (a) 100% sexed semen to raise surplus springers (SS), and (b) sexed and beef semen for adequate replacements plus crossbred calves (XB). We estimated breedings to sexed semen needed to support a specified herd size and replacement rate (RR) and the number of day-old crossbred calves if the remaining were to beef semen. Young-stock NPV was stochastically modeled under each scenario, accounting for animal sales (day-old dairy bull calves and crossbred beef calves, surplus springing heifers) and raising costs. Springing heifer sales and costs by stage were discounted at 5% annually relative to day-old calves. The 2 strategies were evaluated for several herd size-RR scenarios with otherwise identical inputs for non-completion and reproductive parameters. The present value cost to raise a replacement heifer to 24 mo was \$3.05/d (\$2,233) and each additional (marginal) heifer was \$1.79/d (\$1309). The price per head for animals sold was \$400 (dairy bull calves), \$750 (beef cross calves, range \$350–\$1,000), and \$2,300 (springers, range \$1,500–\$3,000). In each herd size-RR scenario, the SS strategy was more costly than XB on average and in 78% of iterations. For a 3,000-cow herd with 35RR, the mean lost revenue (opportunity cost) of the SS strategy was \$288K (range \$871K loss to \$297K gain) versus XB. The XB strategy was increasingly more profitable relative to SS at lower RRs and with improved reproduction. There were 1.3 surplus XB animals to sell for every 1 SS; the average NPV per head was \$750/XB compared with \$702/SS (weighted average of springers and bull calves). On average, SS was 91% more costly than XB at 25RR, 24% at 35RR, and 12% at 45RR. This analysis indicates the opportunity to benefit from beef semen usage. The highest penalty for only sexed semen is in low RR and high reproduction herds (i.e., they produce many more calves than needed as replacements). This limited analysis does not capture tradeoffs associated with risk management or lactating herd performance.

Key Words: replacement strategy, dairy-beef, discounted net cash flow (NPV)

1265 Optimizing dairy cow replacement decisions by applying reinforcement learning in a stochastic environment. Y. Gong^{*} and V. E. Cabrera, University of Wisconsin–Madison, Madison, WI.

Culling decisions in dairy farming are both complex and crucial for profitability. However, due to uncertainty, lack of decision-support tools, and long-term forecasting challenges, farmers often rely on short-term financial data, leading to suboptimal replacement decisions. To address this issue, we developed a reinforcement learning (RL) framework to optimize dairy cow replacement decisions dynamically. Our approach established a Markov Decision Process as the backbone of RL model, where state variables represent individual cows based on parity (1 to 12), months after calving (1 to 20), months in pregnancy (0 to 9), and disease status (healthy or sick). The model maintains a constant herd size, with replacement heifers entering the herd upon culling. Monthly stochastic processes determine cow events such as pregnancy, disease, and recovery based on literature-derived distributions. Milk production follows parity-specific US Holstein lactation curves, and the economic model incorporates income sources (milk, calf, carcass) and costs (replacement, breeding, disease treatment) based on recent market prices. We used Q-learning, a classic RL algorithm, to train the model over 5 million episodes. Each episode simulated 60 mo in the future, allowing the model to iteratively learn optimal replacement policies based on cow-specific states and expected rewards. Preliminary results demonstrate replacement policies comparable to the current state-of-the-art dynamic programming approach. The net retention value curves show a decreasing value for nonpregnant cows as lactation progresses, with a U-shaped trend where the value increases upon pregnancy. For healthy open cows, the model suggests replacement around 14 mo post-calving for parity 1 and 12 mo for parity 2, while sick cows are culled 2 to 3 mo earlier than their healthy counterparts. These findings align with existing literature, validating RL's potential as a data-driven, farm-specific decision-support tool and offering a promising alternative to traditional methods for improving economic efficiency in dairy herd management.

Key Words: artificial intelligence, dairy cow management, Monte Carlo

1266 Data-driven lactation curve model to quantify milk yield potential and loss in Holstein cattle. A. Wang^{*1}, L. Han², and Y. Wang¹, ¹China Agricultural University, Beijing, China, ²Ningxia University, Yinchuan, Ningxia, China.

Lactation in the dairy cow is a complex physiological process. Traditional empirical models aim to describe mean value with many features of yield loss being ignored. Incorporating disturbances into lactation curve allows for quantification of milk loss and prediction of individual potential, which contributes to precision livestock farming. This study developed a potential lactation curve model (PLCM) and evaluation process based on high-throughput session milk yield records by interpretable mathematical and biological models. The general expression of PLCM is as follows: $YP = YA + YE + YM$, where YP and YA are potential and actual milk yields, respectively; YE is the environmental milk loss; and YM is the potential milk loss due to any unknown reasons. We estimated the environmental loss based on lactation physiology by combining the Wood model with ordinary differential equations. Three processes were considered: variability in the number of mammary gland cells when cows were challenged with various environmental events, milk secretion, and incomplete milking. For potential loss, a linear model was developed by non-negative matrix decomposition and information entropy based on the approximately linear inverse relationship between resilience and yield. A total of 64,574,295 session milk yield records from 47,044 Holstein cattle in China were available from 2020 to 2024 for evaluation. The 305-d actual and potential milk yields were $10,478.68 \pm 2,402.03$ kg and $12,512.19 \pm 2,799.31$ kg, respectively. The environmental and potential milk losses were 826.18 ± 765.68 kg and $1,192.42 \pm 632.57$ kg, which means that 6.60% and 9.53% milk

yields were not fully expressed due to various challenges and individual reasons, respectively. Therefore, it can be inferred that milk yield can be further increased by at least 16.13% through improved management and breeding practices. The PLCM could be a valuable phenotyping tool to provide a framework for assessing the production sustainability and potential of the cow. Further, the extension of PLCM to DHI data allows for a more generalized genetic evaluation of individual potential and loss in dairy cattle, and to gain a more advanced knowledge about production performance.

Key Words: potential milk yield, lactation curve, milk loss

1267 Effect of 2 simulated heat waves on technological properties of cow milk. L. Rey-Cadilhac^{*}, A. Boudon, A. Chauvin, and C. Hurtaud, PEGASE, INRAE, Institut Agro, Saint Gilles, France.

During summer, dairy cows experienced heat stress (HS), impacting their welfare and health but also their productivity and milk quality. While HS impacts milk composition, its effect on technological properties like heat stability and lipolysis remains unclear. These properties cannot be corrected at the plant, making farm-level control crucial. This study aimed to evaluate the impact of heat waves on these properties. Six cows were housed in 2 temperature-controlled rooms with *ad libitum* diet and water. They underwent 1 wk of neutral temperature (19°C), followed by 1 wk of HS (36°C by day, 19°C by night), and 1 wk of recovery at 19°C. This sequence was repeated to simulate 2 consecutive heat waves. Heat stability was assessed using ethanol and Ramsdell tests, while lipolysis was measured by infrared spectroscopy, on the last day of the first week, and at each milking on the first, second, and last day of the 2 following weeks of both repetitions. Milk yield, fat content, and protein content were recorded daily at each milking. A mixed model analyzed separately the variation of these parameters in the morning and evening milkings, considering day, repetition, and room along with their interactions as fixed effects, cow as a random effect, and day within repetition per cow as a repeated measure. Milk yield and protein content decreased during both heat waves but returned to initial levels at the end of recovery period, with a lower amplitude in the second wave for protein content. Ramsdell test showed increased stability during the first 2 d of heat stress (from 1.7 to 1.82 mL KH_2PO_4), decreasing afterward and throughout recovery (until 1.55) ($P_{\text{day}} < 0.05$). Ethanol test results showed similar trends during the first heat wave but no significant variation during the second one. Lipolysis was higher in the evening milk at the end of the first heat wave and for 3 d afterward (from basal level ~ 0.3 – 0.4 to 0.7 mEq/100 g fat), with no significant variation during the second ($P_{\text{dayrepetition}} < 0.001$). Heat waves affected the technological properties of milk, with some variations between repetitions. Further analysis of milk fine composition is ongoing to explain these technological changes.

Key Words: heat stress, lipolysis, heat stability

1268 Investigating the short-term impacts of mid-lactation hoof trimming in multiparous cows on milk production. E. Shepley^{*1}, G. Cramer¹, and S. Van De Pol², ¹University of Minnesota, St. Paul, MN, ²CATTLEytics Inc., Hamilton, ON, Canada.

The current study aims to determine the short-term effect of providing a mid-lactation hoof trimming (HT) on the milk yield of multiparous cows. Second- and third-lactation cows with no history of hoof lesions from 3 farms were enrolled at 99 ± 19 DIM on one farm and 117 ± 2 DIM on the other 2 farms and randomly assigned to receive a mid-lactation HT (TRT; $n = 2,793$), or a negative control (CON; $n = 2,894$) that was not trimmed. Cows were allocated at the beginning of the week they

were due to receive their mid-lactation HT. Only cows in the TRT group were put on the list to receive an HT in the week of enrollment. The enrollment date was considered d 0 in our analysis for both groups. Milk yield data were extracted from a data aggregation platform (MilkShake, CATTLEytics). Milk yield (kg/d) was analyzed using a mixed-effects model, including the fixed effect of treatment, lactation, and average milk yield across the 7 d before hoof trimming, and farm, as well as the repeated measure of day relative to enrollment (d 0 to 14) and the random effect of cow. As enrolled cows were of Jersey, Holstein, or Jersey-Holstein crosses, breed composition (% Jersey, where 0% is full Holstein and 100% full Jersey) was also included as a covariate. The preliminary analysis included 3,924 (TRT = 1,864, CON = 1,994) cows

with complete milk yield data around enrollment. Breed composition (% Jersey), DIM, baseline milk yield, and parity were similar in TRT and CON. There was no evidence of a difference in overall milk yield across the 14 d following enrollment for TRT cows (30.5 kg/d, 95% CI: 30.4, 30.6) compared with CON cows (30.6 kg/d, 95% CI: 30.4, 30.7). Similarly, there was no evidence of differences in milk yield on any of the 14 d following enrollment. The study results suggest that the disruptions on HT day to the life of a multiparous cow with no history of hoof lesions has a limited impact on milk production.

Key Words: hoof health, lameness, production efficiency

Applied Nutrition Symposium: Milk Pricing Dynamics and Strategies for Enhancing Milk Fat Production

1269 Balancing dietary amino acids for optimum milk fat synthesis and implications of dietary fatty acids. M. E. Van Amburgh* and A. C. Benoit, *Department of Animal Science, Cornell University, Ithaca, NY.*

With the implementation of genomic selection, the genetic capacity for milk fat and protein synthesis has dramatically increased. For milk fat, the rate of change is nearly exponential, given the selection pressure on genes and alleles such as SCD1 and DGAT1. These changes are analogous to creating a new genotype similar to reevaluating new generations of growing swine to match the EAA supply to the protein accretion rate of the animal. Data from cell culture and in vivo experiments have demonstrated interactions between AA and fatty acid (FA) supply on milk fat synthesis and yield. To highlight a few, Lys has been shown to facilitate the upregulation of FABP and SREBP1, which in turn upregulates enzymes such as ACS, ACC, and FAS, enhancing de novo FA synthesis. Met and Leu have also been shown to be involved in the upregulation of SREBP1, whereas Arg increased de novo and mixed FA synthesis through increased expression of ACC, SCD, and DGAT1. Elongation of the FA carbon chain requires FAS and data has demonstrated the role of His, Lys, Ser, and Cys in the expression and activity of the enzyme system, also suggesting that FAS has a requirement for both EAA and NEAA. In addition, there are interactions with the amount and profile of FA supplied to the cow. The general recommendation is to feed moderate levels of total FA and a balanced ratio of individual FA similar to balancing for EAA. To achieve increased FA synthesis, the requirements of EAA at a metabolizable level are generally much greater than have been historically supplied. Using the approach of Higgs et al., the grams per Mcal of ME provides a more precise approach for achieving the supplies required to increase the expression of these enzymes. For example, supplying at least 1.19 g metabolizable Met and His/Mcal ME, and 3.2 g metabolizable Lys/Mcal ME provides adequate levels of those EAA to achieve increased milk fat synthesis. It is also important to consider dietary sources of substrates that enhance ruminal butyrate production, as butyrate is used in significant quantities to synthesize FA and triglycerides on a molar basis. To realize the genotypic capabilities of high-producing dairy cattle to yield more milk fat, nutrient supplies need to increase concomitant with the change in capacity.

1270 Optimizing milk fat: Aligning dairy nutrition with genetic progress. A. L. Lock* and J. M. dos Santos Neto, *Michigan State University, East Lansing, MI.*

With the growing emphasis on milk components and driven by shorter generational intervals, genetic selection for milk fat has increased exponentially in the past decade. This has prompted questions about whether nutrition can keep pace with the rapid genetic progress in milk fat. To address this, we must improve our understanding of how the mammary gland makes milk fat, the precursors it requires, and the mechanisms to provide them. We will present recent research that underscores the latest advancements in these areas. Milk fat contains triglycerides with fatty acids (FA) from de novo (<16 carbon FA) and preformed (>16 carbon FA) sources. Mixed FA (16-carbon FA) can be derived from both sources. A balance of de novo and preformed FA incorporation into milk triglycerides is needed for maintenance of milk fat fluidity. A common dietary approach to increase milk fat yield is by increasing the supply of mixed and preformed FA to the mammary gland. Consistent evidence from the

literature underscores the effectiveness of palmitic acid-enriched diets in increasing milk fat yield. Moreover, it emphasizes that responses to different dietary 18-carbon FA will vary. Recent studies have explored the use of oilseeds, with high-producing dairy cows consuming whole cottonseed and roasted high oleic acid soybeans showing promising results. Additionally, sodium acetate supplementation increases milk fat yield by increasing de novo and mixed FA yield. This approach may not be practical in a commercial setting, but it shows the potential for improving milk fat yield by increasing de novo FA substrate supply to the mammary gland. Previously, studies in the literature primarily focused on strategies to increase milk fat yield by enhancing substrates for de novo FA or dietary FA for preformed FA. Recent research, however, has emphasized the importance of increasing the supply of both sources to the mammary gland and the important role played by C16:0. In summary, given rapid increases in genetic selection for milk fat, recent research underscores the need to develop dietary strategies that target increased yields of both de novo and preformed FA in the mammary gland. This approach is crucial to ensure long-term, sustained increases in milk fat production.

Key Words: de novo, milk fat, palmitic acid

1271 The economics of butterfat production. N. R. St-Pierre*, *The Ohio State University, Tangier, VA.*

Until 2000, US milk was paid based on butterfat and solids nonfat. The price support system used underpaid butterfat while overpaying solids nonfat. The 2000 Farm Bill reformed the pricing system using end-product pricing. From then on, butterfat price has been calculated from the unregulated wholesale price of butter. This led to an increase in domestic butterfat price. Questions remain regarding the economics of butterfat production. At the onset, one must separate strategic vs. tactical butterfat production. Strategic production is what would be produced without any stimulants to fat production. Strategic butterfat only needs to consider the nutritional costs associated with a unit of butterfat output. Based on NRC (2001), one pound of butterfat requires 4.25 Mcal of NEL, 0.17 lb of MP, 1.13 lb of effective NDF (eNDF), and 0.38 lb of non-effective NDF (neNDF). The software Sesame uses hedonic pricing to extract the average cost of these 4 nutrients across all feedstuffs traded in a given market. From 2015 to the end of 2024, the average, minimum, and maximum implied prices per unit in the US have been: \$0.10/Mcal NEL (\$0.06 to \$0.18); \$0.30/lb of MP (\$0.10 to \$0.60); \$0.05/lb of eNDF (–\$0.04 to \$0.10); and –\$0.05/lb neNDF (–\$0.20 to \$0.10). During the same period, butterfat price averaged \$2.54/lb (minimum \$1.32, maximum \$3.71, SD \$0.56). Therefore, during this 10-year span, one pound of butterfat has generated an average of \$2.54 in additional gross income, required an average of \$0.52 in nutrient costs, resulting in an average of \$2.02 in net income. Importantly, the price paid for butterfat always exceeded the cost of the required nutrients. The economics of strategic butterfat production is simply a no-brainer. Tactical butterfat is a very different proposition. First, the butterfat response varies not only by the stimulant under consideration (e.g., palmitic acid, methionine analog, and so on), but also by breed, stage of lactation, parity, and other unknown factors. That is, there is uncertainty in the response to the stimulant for a given herd. Decision making under uncertainty must go beyond calculating the average benefits. A detailed example will be provided for palmitic acid supplementation.

Ruminant Nutrition 2: Physiology, Fermentation, and Digestion

1272 Partially replacing soybean meal with sprouted peas or sprouted soybean yielded similar fermentation pattern in a dual-flow continuous culture system. A. C. S. Vicente^{*1}, G. K. Salas-Solis¹, M. L. Johnson¹, C. A. Hammond¹, A. S. R. Fonseca², A. C. da Costa², J. E. P. Santos¹, J. Rohrer³, and A. Faciola¹, ¹*University of Florida, Gainesville, FL*, ²*Universidade Federal do Mato Grosso, Cuiaba, Mato Grosso, Brazil*, ³*RP Nutrients Inc., East Troy, WI*.

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

Peas and soybeans are legumes and rich in phenolic compounds (e.g., tannins), which have shown potential to modify ruminal fermentation. The objective of this study was to evaluate the effects of partially replacing soybean meal (SBM) with sprouted peas or sprouted soybean on ruminal fermentation in a dual flow continuous culture system. Six fermenters were used in a 3 × 3 replicated Latin square with 3 periods of 10 d each. The study evaluated 3 dietary treatments: a control diet (CON) containing 21% SBM; a diet in which 23% of SBM was partially replaced with sprouted peas (SP); and a diet in which 29% of SBM was partially replaced with sprouted soybean (SS). All diets had similar nutritional composition with 17% CP, 30% NDF, 41% NFC, and 4.5% EE (DM basis) and they were fed twice daily, totaling 106 g/d. The liquid and solid passage rate of the in vitro system was regulated to 11% and 5.5% per hour, respectively. In each experimental period, after 7 d of adaptation, samples were collected for 3 d for analyses of ruminal fermentation at 0, 1, 2, 4, 6, and 8 h post-morning feeding. Statistical analysis was performed with the mixed procedure of SAS, data using time points were analyzed using repeated measures. The treatment effects were depicted using multiple comparisons, the *P*-values were adjusted using Tukey correction, and significance was declared when *P* ≤ 0.05. There were no effects of treatment throughout the day for pH (LSM ± SEM: 6.04 ± 0.085); ammonia concentration (16.40 ± 0.606 mg/dL); total volatile fatty acid concentration (83.31 ± 3.492 mM); molar proportions of acetate (54.28 ± 0.861%), propionate (20.48 ± 1.462%), and butyrate (17.05 ± 0.880%); and acetate to propionate ratio (2.74 ± 0.195). These results demonstrate that a partial replacement of SBM with SP or SS in lactating dairy diets may be an alternative to SBM, yielding similar fermentation patterns.

Key Words: alternative protein, legume, pea

1273 Effects of *Aspergillus oryzae* and isoacids on ruminal fermentation, nutrient degradability, and nitrogen balance in a dual-flow continuous culture system. M. L. Johnson^{*1}, C. A. Hammond¹, G. K. Salas-Solis¹, M. U. Siregar¹, A. C. S. Vicente¹, E. Sarmikaso-glou¹, V. Augusto¹, M. Socha², C. Cassady³, and A. Faciola¹, ¹*University of Florida, Gainesville, FL*, ²*Zinpro Corporation LLC, Eden Prairie, MN*, ³*Biozyme Incorporated LLC, St. Joseph, MO*.

Isoacids included in dairy cattle diets have the potential to improve ruminal fiber degradability and nitrogen utilization by stimulating fiber degrading ruminal microorganisms. *Aspergillus oryzae* prebiotics (AOP) have been shown to increase nutrient utilization by stimulating rumen fungal and bacterial populations. The effects of combining isoacids with AOP remain unclear; therefore, the objective of this study was to determine the interaction of including isoacids, AOP, and a blend of the 2 on ruminal fermentation using a dual-flow continuous culture system. We hypothesized that the inclusion of isoacids and AOP would improve nutrient utilization. The experiment was conducted in a 4 × 4

Latin square design with a 2 × 2 factorial arrangement combining the 2 factors: (1) an isoacid blend of isobutyrate and 2-methylbutyrate as well as (2) an AOP (Amaferm). Treatments were randomly assigned as (1) Control (basal diet without the inclusion of isoacids or AOP), (2) ISO (basal diet plus the inclusion of 0.1604% isobutyrate and 0.0660% 2-methylbutyrate), (3) PREB (basal diet plus the inclusion of 0.0169% of AOP), and (4) ISOPREB (basal diet plus the inclusion of both isoacids and AOP). The fermenters were provided with 106 g/d of diets containing 16% CP, 32% NDF, and 30% starch. Each experimental period was 20 d long where the first 17 d were used to adapt the fermenters to each treatment and the last 3 d were utilized to assess pH, NH₃-N, nitrogen metabolism, and nutrient digestibility. Experimental data were analyzed using the MIXED procedure of SAS. There was an ISO × AOP interaction for NH₃-N concentration (*P* = 0.02) where ISO and AOP reduced NH₃-N when included alone, but their combination increased NH₃-N concentration. The AOP increased RUP flow (*P* < 0.01) and reduced RDP flow (*P* < 0.01) and CP digestibility (*P* < 0.01). Our results indicate that the inclusion of isoacids and AOP altered NH₃-N concentration and had no effect on pH, DM, and NDF digestibility. Further analyses will need to be completed to understand the potential effects of isoacids and AOP on volatile fatty acid concentration.

Key Words: NH₃-N, rumen-degraded protein (RDP), rumen-undegraded protein (RUP)

1274 Assessing the interaction between supplemental branched-chain volatile fatty acids (BCVFA) and diet RDP on recovery of ¹³C-labeled BCVFA and ¹⁵N into bacterial AA in continuous cultures of ruminal microbes. A. F. White^{*3}, A. R. Sanders¹, K. E. Mitchell⁴, M. T. Socha², D. H. Kleinschmit², C. Lee¹, and J. L. Firkins¹, ¹*The Ohio State University, Columbus, OH*, ²*Zinpro Corporation, Eden Prairie, MN*, ³*Agri-King Inc., Fulton, IL*, ⁴*Elanco Animal Health, Indianapolis, IN*.

In a previous study, dosing branched-chain volatile fatty acids (BCVFA) increased recovery in bacterial BCAA through reductive carboxylation. We hypothesized that increasing BCVFA supply would interact with RDP supply to influence bacterial protein degradation through expression of peptidase(s), differentially impacting the recovery of ¹³C-labeled BCVFA into bacterial BCAA. Using ¹⁵N as a microbial marker, its enrichment in bacterial AA should increase with decreasing RDP or if dosed BCVFA decreases peptidolysis, as previously shown. Treatments formulated with low or high (8.5% or 10.0% based on NASEM, 2021) RDP and 0, 1.18, or 2.15 mmol/d of each BCVFA were arranged in a 2 × 3 factorial in a 6 × 6 Latin square design. Periods had 7 d of adaptation and 4 d of sampling. Total liquid and solids dilution rates were 10%/h and 5%/h, respectively. Urea was infused for adequate ammonia concentration. Data were analyzed in SAS v. 9.4 PROC MIXED with fixed effect of treatment and random effects of period and fermenter. Contrasts were linear and quadratic doses of BCVFA with low and high RDP, respectively, and the main effect of RDP. Increasing supply of BCVFA increased (*P* < 0.05) the portion of ¹³C recovered in Val and Leu but not Ile. Supplemental BCVFA linearly decreased (*P* < 0.08) the ¹⁵N enrichment of Pro, Phe, and Ile when RDP was adequate but linearly increased (*P* < 0.01) ¹⁵N enrichment of Val and Ala when RDP was limited. When RDP was low, Pro availability may have limited microbial response to BCVFA because of inhibited peptidolysis. When RDP was higher, Ile apparently limited microbial N unless 2-methylbutyrate was dosed at the higher BCVFA treatment; Phe might have been

secondarily limiting. The BCVFA dosing appears to control peptide production and conversion into microbial N, requiring adequate RDP for BCVFA effectiveness. Thus, our results help confirm prior reported results that, if ammonia is not limiting, preformed AA from RDP are needed to optimize effectiveness of supplemental BCVFA to increase fiber digestibility and microbial protein synthesis.

Key Words: isoacid, microbial, amino acid

1275 Recovery of ^{13}C -labeled C from branched-chain VFA (BCVFA), branched-chain amino acids (BCAA), or glucose into bacterial AA in response to RDP and dosing of unlabeled BCVFA in continuous cultures. A. F. White^{*3}, A. R. Sanders¹, K. E. Mitchell⁴, M. T. Socha², D. H. Kleinschmit², C. Lee¹, and J. L. Firkins¹, ¹The Ohio State University, Columbus, OH, ²Zinpro Corporation, Eden Prairie, MN, ³Agri-King Inc., Fulton, IL, ⁴Elanco Animal Health, Indianapolis, IN.

The keto acid of Val (from isobutyrate; IB) can branch to Leu synthesis de novo. Due to this pathway, we hypothesized that interconversion of ^{13}C -IB with its keto acid could label both Val and, to a lesser extent, Leu in low RDP diets. Dosing extra unlabeled Leu was expected to inhibit interconversion. In study 1, treatments with low (8.5%, based on NASEM, 2021) RDP and 1.72 mmol/d of each of the 3 BCVFA without or with supplemental Leu were arranged in a truncated 6×6 Latin square design with 3 periods. The periods had 7 d adaptation followed by 7 d sampling after 2 pulse doses of individually ^{13}C -labeled BCVFA or corresponding BCAA with 4 d of washout between each labeled dose. Data were analyzed in SAS v. 9.4 PROC MIXED with fixed effect of treatment and random effects of period and fermenter. Supplemental Leu did not change ($P > 0.10$) rumen degradation of nutrients or flows of microbial N, but more ($P < 0.05$) ^{13}C was recovered in bacteria from labeled BCAA than BCVFA. In study 2, 4 continuous cultures in 2 periods in a truncated 4×4 Latin square design had low or high (8.5% or 10.0% RDP). Universally ^{13}C -labeled glucose from purified potato starch or purified cellulose was dosed. Enrichment of ^{13}C from either carbohydrate source was not different ($P > 0.15$) from zero (background enrichment) in bacterial AA other than the BCAA, except for Ser, Pro, and Ala for low RDP combined with cellulose. Source of carbohydrate tended to interact ($P = 0.13$) with RDP for ^{13}C enrichment of microbial Val. Microbes fed low RDP synthesized Val from cellulose ($P = 0.06$, above 0) for ^{13}C enrichment, but enrichment of bacterial Val was not different from 0 ($P > 0.15$) for low RDP with starch and high RDP with either starch or cellulose. Enrichment of Ile was numerically decreased ($P = 0.12$) by high RDP but was only >0 ($P = 0.04$) with cellulose and low RDP. Enrichment of bacterial Leu was not affected ($P > 0.15$) by RDP or source of glucose. De novo synthesis of Val is less inhibited than synthesis of Leu by exogenous precursors.

Key Words: amino acid synthesis, rumen bacteria

1276 In situ ruminal degradability of red clover-grass baleage isoflavones in dairy cows fed high- versus low-forage diets. M. A. Arshad^{*}, M. G. M. Carvalho, R. A. Menezes, K. V. Almeida, O. R. Osuolale, M. M. H. Khandakar, W. Sims, M. A. Rahman, and A. F. Brito, *Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, NH.*

Milk concentration of isoflavones is influenced by dietary intake of these phytoestrogen metabolites, but there is limited information about their metabolism in the rumen. We aimed to evaluate the in situ effective degradability (ED) and disappearance of total and individual isoflavones

from red clover-grass mix (RC-GR) baleage in dairy cows fed high-versus low-forage diets. Four ruminally cannulated Holstein cows averaging (mean $\pm$ SD) 214 ± 38.7 DIM and 23.1 ± 1.1 kg/d of DMI in the beginning of the study were used in a crossover design with 2 periods. Each period lasted 16 d with 15 d for diet adaptation and 1 d for samples incubation. Cows were fed 2 experimental diets: (1) 70% forage and 30% concentrate (HF), and (2) 50% forage and 50% concentrate (LF). Approximately 7 g of lyophilized RC-GR baleage samples, containing 1,518 mg/kg DM of total isoflavones, were sequentially incubated in the rumen of each cow in triplicate nylon bags (10×20 cm) for 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, and 20 h on d 16 of each period. Data were analyzed using the MIXED and GLIMMIX procedures of SAS. The concentrations of starch and NDFom averaged (DM basis) 20.8% and 33.4% in the HF diet, respectively, and 24.9% and 28.1% in the LF diet, respectively. The ruminal degradation rate ($k_d = 0.30\%/h$), soluble fraction ($a = 54.6\%$), and potentially degradable fraction ($b = 44.7\%$) of RC-GR total isoflavones were not affected ($P \geq 0.13$) by diets. In addition, diets did not affect ($P \geq 0.26$) the ruminal ED of RC-GR total isoflavones (mean = 93.2%), formononetin (mean = 93.3%), daidzein (mean = 84.9%), and biochanin A (mean = 93.2%). However, the ED of RC-GR genistein tended ($P = 0.08$) to increase with feeding HF (mean = 90.5%) versus LF (mean = 89.0%). The ruminal disappearance of RC-GR total isoflavones (mean = 87.5%) was not affected ($P = 0.16$) by diets. In conclusion, feeding HF versus LF had no effect on the ED and disappearance of RC-GR samples incubated in the rumen of dairy cows for up to 20 h, possibly because the differences in starch and NDFom concentrations between treatments were relatively small.

Key Words: baleage, phytoestrogen, ruminal degradability

1277 Oral swabbing as a non-invasive method for collection of the rumen microbiome: Is timing everything? M. K. Costello^{2,3}, J. C. McClure^{*1}, E. French¹, K. Panke-Buisse¹, and R. Amorin de Hege^{2,1}, ¹USDA-ARS-DFRC, Madison, WI, ²Oakridge Institute for Science and Education, Oak Ridge, TN, ³University of Wisconsin-Madison, Madison, WI.

Traditionally, large numbers of rumen microbiome samples are hard to collect. Recently, oral swabs have been suggested as a noninvasive method to rapidly collect rumen samples. Previous research suggested pre-feeding as the optimal time for swabbing, but did not account for the cow's rumination status during sample collection. This study addresses whether timing of collection and rumination status is important. Multiparous cannulated Holstein cows ($n = 4$, average 163 DIM) were fitted with biometric halters to determine when the cows ate, drank, and ruminated over an 11-d period. Cows were visually monitored for a 24-h period, and an oral swab sample was collected once per hour only when rumination occurred. To reduce the likelihood of differences in the cows' samples due to lactation stage and feed batch, the collection was repeated one week later. A swab was dipped in a mixture of rumen liquids and solids from the cannula as a control. Collected swabs were inspected for color and particulate matter and classified A–E, with A being a dark brown swab with particles, and E, a white swab. DNA extracted from swabs was sequenced on PromethION flow cells using the gDNA Native Barcoding Kit. Average fragment read lengths and standard error (SE) were $4,238 \pm 7$ and $4,999 \pm 7$ bp for oral and cannula swabs, respectively. Percent of bovine reads in oral swabs was calculated, with a minimum of 0.78% and a maximum of 87.6%. Swabs categorized as A had the lowest average percent bovine reads, $12.11\% \pm 0.03\%$ (SE). Fewer than 3 swabs were category D and 0 were category E, but category C and B had $48.86\% \pm 0.5\%$ (SE) and $32.53\% \pm 0.25\%$ (SE), respectively. Three time points were noted when all cows were ruminating during both collections. The

microbial genera with the most classified reads at each time point were *Prevotella* and *Staphylococcus*, with *Prevotella* being the most common across all time points and cows. Preliminary results indicate many of the same microbes are found in swabs across time points, though the number of classified reads may differ. Swab color affected the number of bovine reads from the extracted DNA; therefore, it is imperative to review swab color before DNA extraction.

Key Words: rumen, microbiome, oral swab

1278 Effects of sampling method and diet composition on ruminal fermentation variables in dairy cows. O. Pitkänen^{1,2}, Y. Kim^{*1}, L. F. Martins¹, and A. N. Hristov¹, ¹*Department of Animal Science, The Pennsylvania State University, University Park, PA*, ²*Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland*.

The ororumenal tubing technique (OTT) is an alternative method for rumen sampling that could potentially eliminate the need for rumen-cannulated (RC) cows. However, OTT samples may be unrepresentative and contaminated by saliva. The objective of this experiment was to compare fermentation variables in ruminal samples collected using OTT vs. RC. Six rumen-cannulated Holstein cows were paired according to milk yield (45 ± 8.1 kg/d) and days in milk (145 ± 89 d) and fed low (25% starch and 35% NDF; LS) or high (35% starch and 28% NDF; HS) starch diets in a 2×2 Latin square design experiment with 4 wk experimental periods. Both diets had 16% CP. Cows were fed once daily, and aliquots of RC samples were collected from the reticulum, ventral sack, and caudal and dorsal parts of the feed mat at 0, 2, 4, 8, and 12 h after feeding. Samples using OTT were collected at the same time points as RC samples using a 190-cm-long tube with a 3×12 cm steel probe and a vacuum pump. Data were analyzed with the MIXED procedure of SAS as repeated measures. Diet, period, method, time, diet $\times$ method, and diet $\times$ time were fixed, and cow (square) was a random variable. Average rumen pH (5.90 vs. 6.14; SEM = 0.07) and NH₃ concentration (2.14 vs. 6.23; SEM = 0.41 mM) were lower ($P < 0.01$) for HS compared with LS. Rumen pH was similar at 0 h but lower in HS, compared with LS, after feeding (time $\times$ diet interaction, $P < 0.01$). Rumen NH₃ concentration was greatest for both diets at 0 h and decreased more in LS than in HS after feeding (time $\times$ diet interaction, $P < 0.01$). Overall, dietary effects on rumen NH₃ concentration were equally detected by both sampling methods, but RC detected pH differences better than OTT across time points. Rumen pH was consistently greater ($P < 0.01$) for OTT than RC (7.03 vs. 6.03; SEM = 0.10), likely indicating saliva contamination. Rumen pH was greater ($P < 0.01$) in samples collected from the reticulum and ventral rumen compared with the feed mat (6.13 vs. 5.91 $\pm$ 0.07). In summary, OTT had higher pH than RC samples, but OTT may be used to compare rumen fermentation effects of contrasting diets.

Key Words: rumen sampling, ororumenal tubing, rumen fermentation

1279 Changes in plasma non-esterified fatty acids are correlated with fatty acid absorption in post-peak dairy cows. J. M. dos Santos Neto^{*1}, J. de Souza², and A. L. Lock¹, ¹*Michigan State University, East Lansing, MI*, ²*Perdue AgriBusiness, Salisbury, MD*.

It has been suggested that increased plasma content of nonesterified fatty acids (NEFA) in post-peak cows when fed supplemental fatty acids (FA) is an indicator of negative energy balance and body weight loss. An alternative explanation is that increased plasma NEFA in post-peak cows fed supplemental FA is a consequence of increased absorption of dietary FA. To test this hypothesis, we used a meta-analytical approach

to assess the impact of dietary FA intake, digestibility, and absorption on plasma NEFA content. Our analysis included 325 observations from 8 Michigan State University studies involving 122 post-peak lactation cows (134 ± 45.3 DIM). Diets (% DM) included (mean $\pm$ SD) 29.9 ± 2.38 NDF, 26.8 ± 3.85 starch, 16.9 ± 0.51 CP, and 3.87 ± 0.81 FA. Across treatments, cows averaged 29.5 ± 3.4 kg/d DMI, 46.0 ± 8.1 kg/d milk, 1.69 ± 0.35 kg/d milk fat, and 1.47 ± 0.25 kg/d milk protein. We used mixed models to perform meta-regressions considering random effects of study, period within study, and cow within study. Plasma NEFA content (μ Eq/L) was used as the independent variable and regressed against the intake, digestibility, and absorption of 16-carbon (16-C), 18-carbon (18-C), and total FA (TFA). Increasing the intakes of 16-C ($97.4 \pm 3.0 + 0.04 \pm 0.006x$, $P < 0.01$; RMSE = 14.4) and TFA ($84.2 \pm 6.2 + 0.03 \pm 0.005x$, $P < 0.01$; RMSE = 15.1) linearly increased plasma NEFA, whereas increasing 18-C intake had no effect ($P = 0.97$). Increasing the digestibility of 16-C ($84.8 \pm 13.5 + 0.41 \pm 0.19x$, $P = 0.03$; RMSE = 17.0), 18-C ($72.9 \pm 11.9 + 0.53 \pm 0.15x$, $P < 0.01$; RMSE = 16.6), and TFA ($75.2 \pm 13.9 + 0.52 \pm 0.18x$, $P < 0.01$; RMSE = 17.1) linearly increased plasma NEFA. Increasing the absorption of 16-C ($95.2 \pm 3.0 + 0.07 \pm 0.009x$, $P < 0.01$; RMSE = 14.3), 18-C ($102 \pm 6.8 + 0.02 \pm 0.01x$, $P = 0.05$; RMSE = 16.9), and TFA ($79.4 \pm 6.0 + 0.04 \pm 0.006x$, $P < 0.01$; RMSE = 14.8) linearly increased plasma NEFA. In conclusion, changes in intake and absorption of 16-C, 18-C, and TFA are directly correlated with plasma NEFA content. Increased FA absorption is a more plausible explanation for increases in plasma NEFA compared with negative energy balance and body weight loss when supplemental FA are fed to post-peak cows.

Key Words: nonesterified fatty acid, fatty acid, digestibility

1280 The mechanism of feed efficiency in dairy cows is characterized by a feedback loop between a rumen viral shunt and host anabolic pathways. H. F. Monteiro^{*1}, A. Horst¹, R. Profeta¹, J. Norris¹, C. C. Figueiredo², B. Mion³, C. T. Brown¹, B. C. Weimer¹, J. E. P. Santos⁴, R. S. Bisinotto⁵, E. S. Ribeiro³, F. S. Schenkel³, F. Peñagaricano⁶, E. Kebra⁷, and F. S. Lima¹, ¹*Department of Population Health and Reproduction, University of California–Davis, Davis, CA*, ²*Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University–Pullman, Pullman, WA*, ³*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada*, ⁴*Department of Animal Sciences, University of Florida, Gainesville, FL*, ⁵*Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL*, ⁶*Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI*, ⁷*Department of Animal Sciences, University of California–Davis, Davis, CA*.

Our previous work established an association between the rumen microbiome and feed efficiency in dairy cows, with holobiont models highlighting host-microbiome interactions as key predictors of feed efficiency. However, the mechanisms driving this interaction remain unclear. This study aimed to elucidate how the rumen microbiome contributes to enhanced feed efficiency. Building on findings that efficient cows exhibit elevated ruminal N derivatives and altered protozoal activity, we hypothesized that increased bacterial predation by rumen protozoa shifts microbial populations, influencing N metabolism, ornithine synthesis, and host utilization of this bioactive amino acid. From a cohort of 454 lactating cows phenotyped for feed efficiency, 24 cows (least feed-efficient $n = 12$ cows vs. most feed-efficient $n = 12$ cows) were selected and matched by site, diet, parity, body weight (688 vs. 677 kg; SEM: 34; $P = 0.79$), body energy changes (2.56 vs. 1.76 Mcal/d; SEM: 1.19; $P = 0.52$), and milk production (40.5 vs. 39.8 kg/d; SEM: 2.10; $P = 0.81$) but differed in DMI (26.1 vs. 20.7 kg/d; SEM:

1.05; $P < 0.001$). Rumen content was analyzed for volatile fatty acids, untargeted primary metabolites, 16S and 18S rRNA, and ultra-deep dual-RNA sequencing. Serum concentrations of growth hormone, IGF-1, and insulin were also measured. Statistical differences were considered when $P \leq 0.05$ after FDR correction. Results revealed large viral activity affecting microbial populations across archaea, bacteria, protozoa, and fungi, predominantly driven by ssRNA *Leviviricetes* and dsDNA *Caudoviricetes* phages, along with other dsDNA, ssRNA, and dsRNA phages and viruses. Phylogenomic analysis indicated that mutations in viral maturation proteins or strain specificity may drive microbial and metabolite shifts between the most and least feed-efficient cows. Gene expression analysis in the rumen content showed that feed-efficient cows had increased rumen transmembrane transporter activity, mitochondrial energy synthesis, and epithelial growth pathways, whose cows also had greater ruminal ornithine and serum anabolic hormones, as well as an upregulation of bovine nitric oxide pathway. While it remains unclear whether feed-efficient cows promote viral mutations or acquire the found specific still uncharacterized viral strains, these viral-microbial-host interactions appear to reinforce each other, providing a mechanistic basis for feed efficiency in dairy cattle.

Key Words: genomics, microbiome, virome

1281 Postruminal phytase infusion increases small intestinal phytate disappearance in cattle. R. J. Trotta^{*1}, R. Garcia-Gonzalez², T. Christensen², Y. Dersjant-Li², L. Marchal², and D. L. Harmon¹, ¹University of Kentucky, Lexington, KY, ²Danisco Animal Nutrition & Health (IFF), Oegstgeest, South Holland, the Netherlands.

The objective of this experiment was to evaluate the small intestinal digestibility of postruminally infused phytate in the absence and presence

of exogenous phytase activity. Four Angus $\times$ Holstein steers, fitted with abomasal infusion catheters and ileal cannulas, were randomly assigned to treatments in a 3×4 crossover design. Steers were fed an alfalfa cube/beet pulp pellet diet to minimize dietary phytate. Abomasal infusion treatments were (1) water, (2) 2.5 g/h phytate, or (3) 2.5 g/h phytate + 3,356 units per hour of exogenous bacterial 6-phytase activity (PhyG; Danisco Animal Nutrition & Health). Infusates contained 5% vol/vol Cr-EDTA as a flow marker. Phytate and phytase infusates were kept in separate containers and solutions were continuously infused via Tygon tubing connected through multi-channel peristaltic pumps. Periods were 12 h in length with 12 h for washout between periods. Six ileal digesta samples were collected in hourly intervals, pooled, freeze-dried, and then analyzed for DM, Cr, and phytate. Data were analyzed using SAS for fixed effects of treatment and sequence. Phytate infused was 0.000, 2.38, and 2.43 g/h for water, phytate, and PhyG treatments. Total ileal flow, ileal fluid flow, ileal DM content, and ileal DM flow were not different ($P \geq 0.63$) among treatments. Ileal phytate concentration was greater ($P = 0.004$) for steers abomasally infused with phytate alone because of undetectable phytate in ileal digesta from steers infused with water or PhyG (3.48% of DM vs. 0.000). Postruminal phytate infusion increased ileal phytate flow (3.02 g/h) compared with water and PhyG treatments (0 g/h). Small intestinal phytate digestibility was 0.000%, -27.7%, and 100% for the water, phytate, and PhyG treatments ($P < 0.001$). Under the conditions of the current study, abomasally infused phytate was indigestible in the small intestine. Abomasal infusion of PhyG increased small intestinal phytate digestibility to 100%. These data strongly suggest that cattle have little to no capacity for small intestinal phytate digestion and that exogenous phytase activity increases small intestinal phytate digestibility.

Key Words: phytate, phytase, intestine

Small Ruminant 1

1282 Feed and forage autonomy in dairy sheep systems to face climate change and achieve agroecological transition goals. J. Laclau¹, W. Nasri¹, P. Hassoun², F. Stark², P. Salgado¹, P. Schmidely³, and E. González-García^{*2}, ¹SELMET, CIRAD, INRAE, L'Institut Agro Montpellier SupAgro, University of Montpellier, Montpellier, France, ²SELMET, INRAE, CIRAD, L'Institut Agro Montpellier SupAgro, University of Montpellier, Montpellier, France, ³UMR Physiologie de la Nutrition et Alimentation, INRAE, AgroParisTech, Paris, France.

Current and future challenges imposed by climate change, societal demands and related agroecological transition goals induce systemic redesign discussions in established agricultural and livestock production models. In such context, we aimed to update the state of the art of farming system practices with significant potential to contribute to forage and feed autonomy in dairy sheep farms. To do it, (i) an exhaustive literature review using several sources of information, including scientific and gray literature (n = 308 documents), was combined with (ii) analyzing historic and relevant results obtained in experimentations conducted at the dairy sheep facilities of the La Fage experimental farm (INRAE, France) belonging to the dairy sheep sector in Roquefort (France), and (iii) practical and empirical knowhow were captured from key local stakeholders through semi-structured surveys. A range of potential innovations and the related levers of actions required to improve feeding system practices in dairy sheep farming were identified and discussed. Five major categories of innovations were classified. First, actions at the farming system level, looking to redesign the whole farming system approach for more autonomy and resilience (e.g., modifying farming landscapes with transition from herbaceous grasslands to agroforestry systems). Second, levers influencing the grass and forage system components (e.g., better adapted grass and forage species, more biodiverse grasslands, alternative forage operations and schedules). Third, adding value from alternative, locally available biomass and less-used feedstuffs resources (e.g., crop residues, by-products from the agri-food industry, or unconventional [abandoned?] plant varieties). Animal adaptability (robustness) and feed efficiency, were discussed as potential traits for future breeding programs and as levers at the flock, animal level. Finally, the potential advantages of using precise feeding management practices at the farm level are considered, including the use of interesting sensors and technologies. These strategies were contrasted with farmers in their farms during field visits and discussed with key actors through participatory multi-stakeholder brainstorming.

Key Words: feed autonomy, dairy sheep, innovative solution

1283 Allantois-centesis in late multifetal pregnant ewes to explore the litter partial loss phenomenon. T. Alon^{*1,2}, M. Ross¹, L. Lifshitz¹, G. Kra¹, S. Malitsky³, M. Itkin³, and U. Moallem¹, ¹Department of Ruminant Science, Volcani Institute, Rishon LeZion, Israel, ²Department of Animal Science, the Hebrew University of Jerusalem, Rehovot, Israel, ³Life Sciences Core Facilities, Weizmann Institute of Science, Rehovot, Israel.

The occurrence of partial litter loss (PLL) in ewes with multifetal pregnancies is correlated with litter size: 15%, 35%, 49%, 57%, and 100% in 2, 3, 4, 5, and 6 fetuses, respectively, by an unknown mechanism. The objective was to better explore the mechanism leading to PLL in multifetal pregnant ewes. For this aim, we collected blood samples from 25 ewes at 80, 138, and 143 d in pregnancy (DIP), and collected allantois fluid (ALF) by allantois-centesis using ultrasound (US) guid-

ance from 25 multifetal ewes at 138 and 143 DIP. For ALF collection, the uterus, pregnancy sacs, and fetuses were monitored, and a spinal needle was inserted through the abdominal wall at a 90° angle and advanced until penetrating the uterine wall and fetal membranes. Ten milliliters of ALF was extracted from every single fetus membrane. During the procedure, we determine the fetus's vitality. At lambing, we collected placenta cotyledons from 14 ewes for gene expression and also collected ear tissue samples from lambs for DNA purification to match between ALF and the lambs. The metabolite and hormone data were analyzed using the PROC MIXED of SAS, and the model included the dams' BW and BCS. The gene expression was analyzed with the PROC GLM of SAS. Eleven ewes were categorized as CTL (no fetus loss) and 14 as PLL. No differences between groups were observed in glucose, BHBA, NEFA, lactate, TG, and insulin plasma concentration. The cortisol concentrations were higher in the PLL than in the CTL at 138 DIP (70.3 and 39.7 ng/mL, respectively, $P < 0.01$), with no differences in testosterone. The relative mRNA abundance of genes in cotyledons related to steroids, glucocorticoids, and metabolite transport was determined. The relative gene expression of androgen receptor (AR) was lower in the CTL, SLC2A1 tended to be higher in the PLL, and the NR3C1 tended to be higher in the CTL ewes. Of 85 lambs born, we performed allantois-centesis from 77 fetuses in 25 ewes. Out of the 77, we performed DNA analysis for 42 fetuses (14 dams) and confidently identified 28 lambs that had, in total, 35 ALF samples. We performed metabolomics analysis for the ALF samples, and 338 polar, 388 lipids, and 18 steroids were identified. In summary, differences were observed in plasma cortisol and several placenta gene expression. We will further analyze the metabolomics results to explore potential mechanisms for PLL in ewes.

Key Words: partial litter loss, prolific ewe

1284 Effects of supplementing prickly pear by-products (*Opuntia* spp.) silage in dairy ewes diet on nutritional characteristics of their milk and cheese. R. Gannuscio^{*1}, M. U. Hassan¹, G. Maniaci¹, A. D'Amico², C. Buzzanca², V. Di Stefano², and M. Todaro¹, ¹Department of Agricultural, Food and Forestry Science (SAAF), University of Palermo, Palermo, Italy, ²Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Palermo, Italy.

Prickly pear fruit processing industries produce a significant amount of fibrous by-products rich in bioactive compounds, minerals, and water-soluble carbohydrates. This study explored the potential of using prickly pear by-product silage as a feed for Valle del Belice ewes, aiming to assess its impact on milk and cheese quality characteristics, and the transfer of bioactive compounds to dairy products. The total phenol content (TPC), antioxidant capacity (AOC), and the fatty acid (FA) profiles of milk and cheese were analyzed. A total of 12 ewes (60 d in lactation) were selected and randomly divided into 3 experimental groups homogeneous for parity, live weight, and milk yield. Each group was fed for 14 d (9 d for diet adaptation + 5 d for sampling) with one of the 3 experimental diets based on a Latin square design (3 groups × 3 diets). The diets with the same crude protein and NDF were (1) control (CTR): 900 g/day/head of commercial concentrate + 3.3 kg/day/head of sulla hay; (2) prickly pear peels (PPP): 1.5 kg/day/head of PPP silage (prickly pear peels + 12% wheat bran) + 500 g/day/head of commercial concentrate + 2.7 kg/day/head of sulla hay; (3) peels, pulp, seeds (PPS):

1.0 kg/day/head of PPS silage (prickly pear peels, pulp, seeds + 12% wheat bran) + 500 g/day/head of commercial concentrate + 2.2 kg/day/head of sulla hay. The effect of diet on milk composition was not significant, except for the urea level, which was lower ($P < 0.01$) in the milk produced from PPP and PPS diets than CTR diet. No significant differences were observed between the composition of cheese. The highest TPC levels of milk and cheese were observed in PPP, followed by PPS, while CTR presented the lowest levels ($P < 0.01$). The CTR

milk showed a significantly ($P < 0.01$) higher milk SFA % than PPP and PPS. Moreover, PUFA % was significantly lower in CTR than PPP and PPS milk. Consequently, the UFA/SFA ratio was significantly higher ($P < 0.01$) in PPP and PPS than CTR milk. The same trend in fatty the acidic composition was observed in the cheeses.

Key Words: prickly pear silage, sheep milk, pecorino cheese

Animal Health 3: Calves

1300 Exploring challenges and key outcomes for the diagnosis of respiratory disease in preweaning dairy calves: A mixed-methods analysis. K. Churchill¹, C. B. Winder¹, B. Gohar¹, B. Pardon², and D. L. Renaud¹, ¹*University of Guelph, Guelph, ON, Canada*, ²*Ghent University, Ghent, Belgium*.

Opportunity exists to improve the diagnosis of bovine respiratory disease (BRD), specifically that requires antimicrobial treatment (i.e., pneumonia) in preweaning dairy calves. A gold-standard case definition can be established by leveraging the validated process of developing a core outcome set (COS; i.e., establishing key diagnostic outcomes). The objective of this study was to interview global research experts, selected based on having at least 25 relevant publications in the past 10 years, to rank diagnostic methods and outcomes, suggest additional outcomes, and identify priority challenges for diagnosis. Interviews were recorded and transcribed, and a mixed-methods approach was used for analysis. All 12 researchers unanimously considered lung ultrasound the most accurate diagnostic method. While most acknowledged clinical scoring systems as a faster diagnostic tool, many highlighted their limited value due to suboptimal accuracy. When asked to prioritize diagnostic outcomes from the literature, all researchers ranked lung consolidation (100%) as the top priority, followed by cough (59%), with a preference for spontaneous (42%) over induced (17%). Body temperature (58%), nasal discharge (58%), and resting respiratory rate (50%) were also frequently noted. Additionally, researchers suggested 8 other outcomes for inclusion in the draft COS. Flagged priority challenges for diagnosis that the COS should account for included the need for clear, standardized instructions to ensure consistent calf assessment and the importance of contextual factors (e.g., climate, facility type, and nearby calf comparison). Further, experts also highlighted the value of combining lung ultrasound with clinical scoring systems to refine diagnosis and treatment decisions. The study results indicate global expert consensus for current challenges and key diagnostic criteria for BRD requiring antimicrobial treatment in preweaning dairy calves.

Key Words: respiratory disease, diagnosis

1301 Changes in BLV antibody status of dairy calves aged 0 to 6 months. O. Willoughby¹, E. I. Morrison¹, F. van der Meer², D. Kelton¹, K. L. Spence¹, and S. J. LeBlanc¹, ¹*Department of Population Medicine, University of Guelph, Guelph, ON, Canada*, ²*Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada*.

Bovine leukemia virus (BLV) is highly prevalent in North American dairy cattle. In calves, seropositivity may reflect infection with BLV or colostral transfer of antibodies without infection. The prevalence of BLV in calves and possible transmission between youngstock is not well understood, therefore, this observational longitudinal study aimed to assess changes in antibody status of calves over time. In total, 586 Holstein heifer calves on 9 commercial dairy farms in Ontario, Canada, were enrolled on each farm every 21 d and re-sampled every 21 d until they were 6 mo old. All Holstein heifer calves born between visits were enrolled. Twenty-seven calves were excluded because they died or were sold before 6 mo of age. The mean age at first sample was 15 d and the mean number of samples per calf was 9. Serum ELISA was used to measure antibodies to BLV; seropositivity was defined as $\geq 45\%$ inhibition, seronegativity was defined as $< 35\%$ inhibition, and results in between defined as suspicious. Based on their antibody status over the first 6 mo of life, calves were grouped into 5 categories: always BLV

positive, always BLV negative, became BLV positive, became BLV negative, or other. The frequency distributions for each category were compared across farms using Pearson's chi-squared test. Overall, 58% of calves were seropositive for BLV at their first sample. In total, 50% of calves that were seropositive at their first sample became seronegative, whereas 39% were always seronegative. Across the entire sampling period, 6% of calves were always seropositive. Five percent of calves were classified as other due to crossing the thresholds for positivity/negativity multiple times or having a first or final value classified as suspicious. At 6 mo old, 7% of calves were seropositive. The distribution of calves within these categories varied widely between farms; 13% to 79% of calves changed from seropositive to seronegative ($P < 0.01$), and between 7% and 83% were consistently seronegative ($P < 0.01$). Based on these results we believe early life seropositivity is likely attributable to maternal antibodies against BLV acquired via colostrum. Further testing using RT-qPCR to detect BLV pro-virus will confirm if the apparent positives are "true" infections. This research will contribute to understanding BLV transmission in dairy calves and contribute to the development of a control program for BLV.

Key Words: animal health, disease

1302 Associations of oral temperature with disease outcomes and inflammation in preweaning dairy heifers. K. R. K. Gottwald¹, J. A. A. McArt¹, A. S. Sipka¹, T. Bhattacharjee², and T. E. von Königslöw¹, ¹*Department of Population Medicine and Diagnostic Science, Cornell University College of Veterinary Medicine, Ithaca, NY*, ²*Department of Computer Science, Cornell University, Ithaca, NY*.

Early detection of diarrheal and respiratory diseases is critical for reducing their impact on preweaning dairy calves and improving animal welfare and performance. Body temperature changes can indicate illness or inflammation, and previous work has shown that oral temperature (OT) reliably detects fever in preweaning dairy heifers. Our objectives were to evaluate the relationships between OT, health outcomes, and inflammatory biomarker concentrations. This prospective cohort study was conducted at a single commercial dairy farm in central New York where calves were housed in indoor group pens of 20 calves with free access to water, starter pellets, and non-salable milk fed via an automated milk feeder. Female Holstein calves ($n = 150$) were enrolled at birth and followed through 28 d of life. At 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, and 28 d of life, health assessments were conducted using the University of Wisconsin–Madison Calf Health Scoring App, and oral and rectal temperatures were measured simultaneously with digital probe thermometers. Blood samples (10 mL) were collected at 2, 8, 16, and 24 d of age, with an additional sample collected each week from calves if extreme rectal temperatures were observed ($< 37.1^\circ\text{C}$ or $> 39.5^\circ\text{C}$). Blood samples ($n = 550$), selected to balance for age and health status, were analyzed for IL-10, tumor necrosis factor (TNF), and IFN- γ . Linear mixed effects models were used to evaluate the associations between OT and diarrheal and respiratory diseases. Results indicated that both age and respiratory disease were associated with increased OT, whereas diarrhea was associated with decreased OT (all $P < 0.01$). Interleukin-10 was negatively associated with OT ($P = 0.03$), whereas IFN- γ was positively associated with OT ($P = 0.04$). Tumor necrosis factor showed no association with OT ($P = 0.57$). In summary, a similar relationship between inflammation and OT was observed as is known to exist with rectal temperature. Thus, our findings suggest that OT might be a useful tool in monitoring health of preweaning dairy calves.

Future work should explore diurnal and feeding-related differences in OT patterns, and patterns throughout the course of disease.

Key Words: oral temperature, calf health, cytokine

1303 Evaluating the association between calf health scores and disease detection. B. Xu*, K. R. K. Gottwald, and T. von Königslow, *Department of Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY.*

Bovine respiratory disease (BRD) and diarrhea are major health concerns in preweaning dairy calves. Standardized health scoring systems, such as those from the University of Wisconsin–Madison (UWM), aid in disease diagnosis. However, their accuracy in identifying diseased calves may vary depending on housing conditions, observer experience, and training. This study evaluated the relationship between health scores and treatment data to identify key indicators used by farm staff managing calves in group housing. Female Holstein calves ($n = 150$) from a single New York dairy were monitored from 1 to 28 d of life. Health scores were measured at 1, 2, 4, 6, 8, 10, 12, 14, 18, 22, and 28 d. The BRD and diarrhea cases were identified using the UWM criteria. Treatment records were used as a proxy for morbidity as determined by farm staff and included the assessment of calf activity and feeding behavior. A total of 1,941 assessments were collected, each representing an evaluation of health score and treatment record (881 healthy, 363 BRD, 514 diarrhea, and 183 “other” health events). Analysis was conducted in Python using a Tabular Neural Network (TabNet) classifier. The dataset was split into 80% training and 20% testing with stratified sampling. Feature importance was determined using TabNet’s built-in selection and permutation importance to identify key health score contributors. The model achieved 87% accuracy, precision, and recall for diarrhea (96% and 95%) and BRD (81% and 75%), respectively. Fecal score was the most influential feature, followed by nasal discharge and attitude. Correlation analysis revealed that nasal discharge had the strongest association with BRD detection fecal score with diarrhea detection, and navel condition with other health conditions. Eye and ear scores showed moderate association with BRD detection, and rectal temperature and joint had weaker associations with disease detection. These results highlight the importance of visible health indicators for disease detection in group housing supporting the need for automated surveillance using computer vision, and AI.

Key Words: bovine respiratory disease (BRD), diarrhea, disease detection

1304 Health indicators associated with growth and body weight at slaughter in non-replacement Holstein calves. H. M. Goetz*, A. Keunen², and D. L. Renaud¹, *¹Department of Population Medicine, University of Guelph, Guelph, ON, Canada, ²Mapleview Agri Ltd., Palmerston, ON, Canada.*

The objective of this cohort study was to investigate predictors of body weight (BW) at slaughter in non-replacement dairy calves. Beginning at arrival to a commercial calf-raising facility in southwestern Ontario, Canada, male Holstein calves ($n = 281$) were examined twice daily for fecal consistency and signs of BRD and weighed weekly for 84 d until moving to a finishing facility where they were raised for a further 107 to 248 d (median 137 d). Live weight (LW) and dressed weight (DW) at slaughter as well as days on feed (DOF) were recorded. Calves were categorized into 3 groups based on days with abnormal fecal and respiratory scores: those with no abnormal scores and the remaining

calves split at the median number of days with abnormal scores. Mixed linear regression models were used to identify variables associated with LW, DW, and DOF, with BW at arrival included as a fixed effect and finishing facility as a random effect. Most calves had an abnormal fecal score (58.4%) and 42.0% had an abnormal respiratory score at least once at the starter facility. The median (range) live weight was 330.41 kg (290.47 to 405.32 kg), whereas the median (range) dressed weight was 190.08 kg (158.30 to 220.90 kg). Calves with ≥ 4 d abnormal fecal scores had a lower LW at slaughter than calves with 0 d abnormal fecal score at the starter facility (-8.64 kg, $P < 0.01$, 95% CI = -14.63 to -2.65). Additionally, calves sourced from drovers had higher LW than calves sourced from auction (12.67 kg, $P < 0.01$, 95% CI = 4.72 to 20.63). Similarly, calves with ≥ 4 d abnormal fecal scores had a lower DW (-4.71 kg, $P < 0.01$, 95% CI = -7.98 to -1.44) than calves did not have an abnormal fecal score, and calves sourced from drovers had higher DW than calves sourced from auction (6.91 kg, $P < 0.01$, 95% CI = 2.57 to 11.24). As for DOF, calves with > 2 d with an abnormal respiratory score had +8.48 DOF ($P < 0.01$, 95% CI = 2.68 to 14.28) compared with calves that did not have abnormal respiratory score at the starter facility. Further, for every 1 kg increase in BW on arrival to the starter facility, DOF decreased by 0.77 d ($P < 0.01$, 95% CI = -1.34 to -0.20). These findings show that calf health early in life can affect BW at slaughter.

Key Words: surplus calf, morbidity, dairy-beef

1305 Association of severe diarrhea and BRD with ADG, feed intake, milk replacer refusal and mortality, in preweaning Holstein and BxD calves. D. Morais Moreira*, C. Rocha, R. Aguirre, L. Van Dyk, M. A. Ballou, and V. Machado, *Texas Tech University, Lubbock, TX.*

The objective of this study was to determine the association of diarrhea and bovine respiratory disease (BRD) with average daily gain (ADG), milk replacer refusal (MRR), starter intake (SI), and mortality of Holstein (H) and beef-on-dairy (BxD) calves during the first 56 d of life. A total of 240 newborn calves (114 H and 126 BxD) were enrolled between September 2023 and March 2024, and were housed individually for 56 d, bottle-fed with 6 L/d of milk replacer and had libitum access to calf starter and water. Diarrhea was defined as the presence of watery feces accompanied by dehydration, while BRD was based on the presence of clinical signs such as cough, eye discharge, abnormal respiration, nasal discharge, ear droop or head tilt, and rectal temperature $\geq 39.2^\circ\text{C}$. Both diseases were evaluated by the research team. Body weight was assessed at enrollment and 56 d later. Survival analyses and linear regression models were fitted to the data. The associations between BRD and diarrhea with average daily gain were conditional to breed ($P < 0.05$). Among BxD, the ADG (95% CI) was 0.42 (0.36–0.48) and 0.60 (0.56–0.63) kg/d for calves with and without diarrhea, while that for H calves, the ADG (95% CI) was 0.45 (0.39–0.52) and 0.51 (0.49–0.55) kg/d for calves with and without diarrhea. Additionally, a decrease in ADG associated with BRD was only observed among H calves (BRD = 0.44 kg/d [95% CI: 0.39–0.49], no-BRD = 0.53 kg/d [95% CI: 0.49–0.57]; $P < 0.01$), as no differences were observed among BxD calves ($P = 0.60$). Milk replacer refusals were greater for calves diagnosed with BRD ($P = 0.05$), but the association between diarrhea and MRR was conditional to breed ($P = 0.02$). The difference was greater for BxD calves (2.7 L; $P < 0.01$) than it was for H (1.25 L; $P = 0.01$). Diarrhea and BRD were both linked with decreased starter intake throughout the study ($P < 0.05$), but only diarrhea was associated with increased mortality ($P < 0.01$). Our findings suggest that the association of BRD and diarrhea

in some outcomes related to performance of preweaning calves may be conditional to breed.

Key Words: diarrhea, bovine respiratory disease (BRD), starter intake

1306 Effects of weaning age on health and hematological measures in dairy and dairy × beef crossbred calves. M. P. Pinkerton^{*1}, R. C. Neves¹, J. P. Boerman¹, H. W. Neave¹, E. M. Schafer¹, and J. A. Pempek², ¹Purdue University, West Lafayette, IN, ²USDA-ARS Live-stock Behavior Research Unit, West Lafayette, IN.

Weaning non-replacement dairy calves early may save costs, labor, and time; however, the subsequent stress response may be immunosuppressive, compromising calf health and welfare, and differentially impact dairy and dairy × beef crossbred calves. The objective of this study was to determine the effects of weaning age (6 vs. 8 wk) on health and hematological parameters in dairy and dairy × beef crossbred calves. In 2 replicates, 32 bull calves (n = 16 Holstein [PB] and 16 Angus × Holstein crossbreds [CB]), blocked by initial body weight and breed, were randomized to be weaned at 6 (EW) or 8 wk (CW) of age. Calves were fed 2.8 L of milk replacer twice daily; 7 d before weaning, the morning feeding was removed. Calves were housed in individual hutches with ad libitum access to a texturized calf starter. Calves were assessed daily for clinical signs of diarrhea and respiratory disease (BRD). Blood was collected weekly from wk 4 to 8 and analyzed for hematological outcomes. Hematological parameters were analyzed using a repeated measures ANOVA (PROC MIXED) with calf as the experimental unit; models included the fixed effects of treatment, breed, time, and their interactions, random effect of calf, and week as a repeated measure. A Poisson regression model (PROC GENMOD) was used to test differences between groups in the incidence of diarrhea from wk 4 to 8. There was no significant effect of treatment or breed on neutrophil:lymphocyte ratio, hematocrit, or counts of white blood cells, lymphocytes, red blood cells, monocytes, eosinophils, basophils, or platelets. There was no significant difference in the frequency of diarrhea in EW vs. CW calves (rate ratio: 0.96; 95% CI: 0.56–1.66; $P = 0.89$) or PB vs. CB calves (RR: 1.02; 95% CI: 0.66–1.56; $P = 0.94$). From wk 4 to 8, frequency of BRD was low, with 3 d and 2 d of BRD signs in EW vs. CW and 4 d and 1 d in CB vs. PB, respectively. While these results do not suggest early weaning negatively impacts calf health, a larger sample size may clarify this relationship. Disease risk may increase for non-replacement calves with failed transfer of passive immunity that are weaned early.

Key Words: dairy calf, health, weaning age

1307 Evaluation of the efficacy of an evidence-based algorithm for antimicrobial treatment of neonatal calf diarrhea. L. S. Zakia^{*1}, M. E. Brander¹, D. E. Gomez¹, P. D. Constable², S. J. LeBlanc¹, and D. L. Renaud¹, ¹University of Guelph, Guelph, ON, Canada, ²University of Illinois, Urbana-Champaign, IL.

Neonatal calf diarrhea is a major cause of mortality and economic loss, often leading to antimicrobial drug (AMD) use. However, current AMD treatment guidelines rely on expert opinion or studies from referral hospitals rather than field-based evidence. The objective of this study was to evaluate the effectiveness of an evidence-based algorithm (EBA) for AMD treatment of neonatal calf diarrhea. A blinded, randomized controlled trial compared 2 treatments: (1) an EBA, where calves received AMD (trimethoprim-sulfadoxine 16 mg/kg q12 h for 3 d) only if they showed 2 or more of the following abnormalities: rectal temperature $>38.8^{\circ}\text{C}$ 12 h after anti-inflammatory medication (cut off

based on a prior study), inability to stand, absent suckle reflex, sunken eyes, or scleral injection; and (2) a control group (CG), where all calves received the same AMD at diarrhea onset. All calves received enteral fluids for the duration of the diarrhea and meloxicam (0.5 mg/kg) at the onset of disease. Cox survival analysis, linear and logistic regressions, mixed-effects models, and negative binomial models were used to assess diarrhea duration, mortality, weight gain, treatment for respiratory disease, and number of days with clinical signs of respiratory disease. Diarrhea duration was not significantly different between groups (CG HR: 0.78; 95% CI: 0.52 to 1.16; $P = 0.22$). There were no differences in respiratory disease treatment (CG vs. EBA OR: 1.46; 95% CI: 0.65 to 3.25; $P = 0.92$), days with clinical signs of pneumonia (CG vs. EBA IRR: 1.18; 95% CI: 0.60–2.32; $P = 0.57$), weekly body weights (CG vs. EBA β : -0.46; 95% CI: -3.44 to 2.52 kg; $P = 0.76$), or average daily gain (CG vs. EBA: -0.00; 95% CI: -0.10 to 0.09 kg; $P = 0.96$). However, control calves required more rescue therapy with a secondary AMD (OR: 6.34; 95% CI: 1.28 to 62.06; $P = 0.02$) and tended to have greater odds of mortality (OR: 3.91; 95% CI: 0.90 to 24.03; $P = 0.07$) than EBA calves. Diarrhea duration, weight gain, and respiratory disease incidence were not different with EBA than systematic use of AMD. However, the EBA was associated with a reduced need for rescue therapy and tended to lower risk of mortality, indicating potential improvements in calf outcomes while reducing the use of AMD.

Key Words: antimicrobial drug (AMD), scours, treatment

1308 Evaluating the utility of a digital postmortem to determine the cause of death in preweaning dairy calves. S. G. Umaña Sedó^{*1}, C. B. Winder², H. M. Goetz², J. Caswell², E. Borkowski², J. Mee³, and D. L. Renaud², ¹Virginia Tech, Blacksburg, VA, ²University of Guelph, Guelph, ON, Canada, ³Teagasc, Cork, Ireland.

Postmortem examination (PME) is essential for identifying disease processes, yet uptake of this tool in preweaning dairy calves remains low. While digital PME have been validated in feedlot systems, they have not been validated for dairy calves. The objective of this pilot study was to evaluate the accuracy of a standardized digital post-mortem method for determining cause of death in preweaning calves. Twenty-four non-replacement preweaning dairy calves from calf rearing facilities that died on-farm were transported to the University of Guelph for a complete gross PME by a board-certified pathologist (gold standard). A standardized set of 25 photographic images was taken during each PME, and 4 veterinary practitioners independently reviewed the images, along with the signalment, identifying both affected body systems and a diagnosis. Agreement between the digital PME method and the gold standard was assessed using Cohen's kappa. The pathologist identified an abnormality in 42% of lungs (e.g., bronchopneumonia, pleuritis), 21% of forestomachs and abomasa (e.g., abomasal bloat, abomasal ulcer), 38% of intestines (e.g., enteritis, colitis), and 33% of other organs (e.g., omphalitis, nephritis). A disease process involving the whole body (e.g., septicemia, dehydration) was found in 13% of calves. Agreement between veterinarians and the pathologist varied by system, with median (range) kappa values of 0.56 (0.35–0.72) for intestines, 0.42 (0.15–0.79) for other organs, 0.28 (0.18–0.58) for forestomachs and abomasa, 0.28 (0.06–0.53) for lungs, and 0.02 (-0.1–0.1) for whole-body conditions. Corresponding percent agreements were 78.8% for intestines, 72.5% for other organs, 70.2% for forestomachs and abomasa, 59.8% for lungs, and 38.2% for whole-body conditions. These preliminary findings suggest fair to moderate agreement for most organ systems, but low agreement in whole-body conditions, highlighting the need for refinements in image collection, disease classification, and diagnostic training. Future

research should focus on improving diagnostic accuracy and practical implementation of digital PME methods in calf health surveillance.

Key Words: calf mortality, digital necropsy, postmortem diagnosis

1309 Development of an antibiogram report for bovine respiratory disease: Factors affecting culture-positivity, antimicrobial susceptibility, and multi-drug resistance of 2 pathogens in California dairies. H. F. Monteiro^{*1}, A. H. Jaramillo¹, A. Garzon¹, H. M. Fritz², C. C. Miramontes¹, I. M. Matos¹, T. W. Lehenbauer^{1,3}, S. Aly^{1,3}, F. S. Lima¹, and R. V. Pereira¹, ¹Department of Population Health and Reproduction, University of California–Davis, Davis, CA, ²California Animal Health and Food Safety Laboratory, University of California–Davis, Davis, CA, ³Veterinary Medicine Teaching and Research Center, University of California–Davis, Davis, CA.

Bovine respiratory disease (BRD) remains a costly challenge to the dairy industry, including increased antimicrobial resistance over the years. In human medicine, cumulative antimicrobial susceptibility testing (CAST) is used to generate antibiograms that support empirical decision-making for antimicrobial treatment. The goal of this study was to evaluate a CAST framework for BRD in dairy cattle. We hypothesized that common factors such as age group, season, and farm management would increase the odds of culture-positive, antimicrobial susceptibility, and multi-drug resistance (MDR) of *Pasteurella multocida* and *Mannheimia haemolytica*. A repeated cross-sectional study was conducted in 3 dairy farms in California, where calves, heifers, and cows were sampled at least monthly for 2 years using deep nasopharyngeal swabs (n = 301). Antimicrobial susceptibility testing was performed on recovered isolates (n = 180) using the minimum inhibitory concentration (MIC) approach for 12 antimicrobial drugs of relevance to dairy cattle. Statistical analyses were performed using a generalized linear model in SAS. An interaction between age and season was observed affecting the odds of culture-positive of both pathogens, whereas farm affected the odds of culture-positive of *M. haemolytica*. Except for penicillin, the odds of nonsusceptible *P. multocida* across tested antimicrobials were increased due to farm management, which also was observed for tilmicosin and tetracycline in *M. haemolytica*. Calves and heifers had the highest odds of nonsusceptible *P. multocida*, while for *M. haemolytica* this was only observed for heifers. Farm was the main factor associated with MDR, with one farm accounting for over 90% of all *P. multocida* isolates and 100% of *M. haemolytica* isolates presenting MDR. A CAST framework adapted for BRD in dairy cattle revealed that farm management, age, and season can significantly influence antimicrobial treatment decisions, highlighting the need to consider these factors when collecting data and presenting CAST reports.

Key Words: pathogen surveillance, antimicrobial stewardship, antibiotic

1310 Comparison of virulence and resistance genes in *Mannheimia haemolytica* and *Pasteurella multocida* from dairy cattle with and without bovine respiratory disease. A. Garzon^{*1}, C. Miramontes¹, B. C. Weimer^{1,2}, R. Profeta^{1,2}, A. Hoyos-Jaramillo¹, H. M. Fritz³, and R. Pereira¹, ¹Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA, ²100K Pathogen Genome Project, School of Veterinary Medicine, University of California, Davis, CA, ³California Animal Health and Food Safety Laboratory, University of California, Davis, CA.

Mannheimia haemolytica (MH) and *Pasteurella multocida* (PM) are 2 of the main bacterial pathogens associated with bovine respiratory disease (BRD). Bovine respiratory disease represents one of the most significant health challenges in the cattle industry, causing substantial economic losses through animal morbidity and mortality while raising serious welfare concerns. The objectives of this project were to (1) characterize virulence factors (VF) and antimicrobial resistance (AMR) genes in MH and PM isolates from BRD-affected and healthy dairy cattle of different ages using isolate whole-genome sequencing (WGS); (2) evaluate associations between these genetic elements and disease status; and (3) assess the accuracy of genome-based predictions for antimicrobial resistance phenotypes. This case-control study determined AMR and virulence factors from 149 PM and 68 MH isolates collected using a deep nasopharyngeal swab from preweaning calves, weaned heifers, and cows with and without BRD. Twenty AMR genes for 10 antimicrobial classes were identified in PM isolates, whereas 11 AMR genes for 7 antimicrobial classes were identified in MH isolates. Additionally, 28 and 15 virulence genes were identified in PM and MH, respectively. The large genetic diversity observed in both bacterial species prevented the identification of unique genetic markers associated with disease status or age group. The ability of WGS-based predictions to predict phenotypic antimicrobial resistance showed variable accuracy across different antimicrobials, achieving moderate levels of agreement overall. Findings from this project demonstrate that identifying genomic markers based on gene presence/absence lacks discriminatory power within this population for identifying unique genotypes associated with disease status in these genomically diverse organisms. Further research incorporating a larger sample of bacterial isolates from both BRD and healthy animals would provide better insight into bacterial genetic diversity and help identify specific genomic signatures associated with disease status.

Key Words: dairy cattle, antimicrobial resistance, whole-genome sequencing

Breeding and Genetics 1: Statistical Genomics in Dairy Livestock Breeding

1311 Modeling production and environmental metric changes between genomic and traditional selection methods. A. L. Herick^{*1}, M. I. Marcondes^{1,2}, J. S. Neibergs³, J. Dalton⁴, and H. L. Neibergs¹, ¹*Department of Animal Science, Washington State University, Pullman, WA*, ²*William H. Miner Agricultural Research Institute, Chazy, NY*, ³*School of Economic Sciences, Washington State University, Pullman, WA*, ⁴*Department of Animal, Veterinary, and Food Sciences, University of Idaho, Caldwell, ID*.

One of the leading causes of changes occurring in the dairy industry is the introduction and adoption of genomic selection. Genomic selection increases selection accuracy and decreases generation interval, accelerating the rate of genetic change and leading to improved farm profitability and production efficiency. The aim of the study was to estimate farm profitability, production metrics, and environmental emissions across 3 selection scenarios: scenario 1 (traditional selection), scenario 2 (single trait genomic selection), and scenario 3 (multiple trait genomic selection). Herd metrics were estimated using a Markov chain model, where herd profitability was maximized and evaluated a dairy achieving a steady-state over 10 years. Herd constraints included heifer and cow culling rates, operation costs (labor, diets, and breeding), and prices for cull animals and calves. The herd metrics from the 10th year were used to evaluate the scenarios. All inputs of the model reflected a Holstein herd and used increased progress values for fluid milk (scenarios 2 and 3) and pregnancy rates (scenario 3), to reflect the impact of genomic selection. Using scenario 1 as a baseline for comparison, dairy profit increased 50% in scenario 2 and 64% in scenario 3. These increases in profit are primarily attributed to increased fluid milk production and more efficient reproduction. Scenario 3 showed an increase in heifer pregnancy rate of 25% and 30% in cows, compared with scenarios 1 and 2. Environmental metrics were evaluated to estimate efficiency improvements. Examining these metrics by per unit of output, methane production per liter of milk was 7% and 6% less in scenarios 2 and 3, respectively. Phosphorus was reduced by 13% in scenario 2 and 8% in scenario 3 compared with the baseline. Nitrogen was similarly reduced by 15% and 11% in scenarios 2 and 3, respectively. The increase in efficiency highlights opportunities for producers to meet growing demands for improved sustainability, using genomic selection by establishing continued progress through generations of improved breeding.

Key Words: genomics, modeling, efficiency

1312 Note on binning a continuous trait: A phenotypic analysis perspective. X. Wu^{*1}, A. Horn², E. Nicolazzi¹, and J. Cole¹, ¹*Council on Dairy Cattle Breeding, Bowie, MD*, ²*Brown Swiss Association, Beloit, WI*.

Grouping continuous phenotypic traits into discrete grades or scores has been a longstanding practice in dairy genetic evaluations and management, facilitating the ease of interpretation and decision making. However, the protocols are *ad hoc*, varying with various breeds and breed associations. It inherently leads to information loss, and optimal strategies for binning continuous phenotype measurements remain underexplored. This paper addresses the relevant principles and methods from a phenotypic analysis perspective, focusing on methodological challenges and optimal strategy. We compared 2 commonly used methods, equal-interval (EI) and equal-percentile (EP), highlighting their strengths and weaknesses, and derived optimal interval sizes for EI-binning. The

mismatch between the bin structure and the data distribution leads to a loss of both within-bin and between-bin rankings. EI-binning often results in a mix of dense and sparse bins, leading to significant within-bin rank loss in densely populated bins. In contrast, EP-binning is robust to distribution shapes because they adjust bin boundaries to ensure equal-frequency representation. However, it can substantially increase the “phenotypic variance” as the number of groups increases. EI retains more information for identifying individuals in the tails of the distributions, such as the tallest or shortest animals. In contrast, the EP approach better preserves rank correlation by distributing individuals equally across groups, favoring applications where intermediate phenotypes are prioritized. Results suggest that 20 to 50 groups provide optimal concordance and minimal information loss, while 5 to 10 groups offer a practical compromise between simplicity and accuracy. This paper also demonstrates how shifting phenotype distributions, as observed in stature over time, necessitate periodic re-evaluation of scoring systems to minimize information loss and maintain concordance. This study provides actionable insights for improving current discretization practices, ensuring robust genetic evaluations, and informing future updates to scoring systems in the dairy industry.

Key Words: dairy cattle, information loss, rank correlation

1313 Simulation analysis of breeding strategies for sub-Saharan Africa dairy cattle production. S. Gebeyehu^{*1}, B. J. Heins¹, and T. Sonstegard², ¹*University of Minnesota*, ²*Acceligen*.

Sub-Saharan Africa (SSA) dairy systems predominantly rely on local breeds with lower milk production potential; however, they play a significant role in the region’s economy. Exotic breeds introduced to increase milk production in the region often underperform due to low heat tolerance and disease resistance ability. The project focused on multiplex gene editing for heat tolerance, resistance to tuberculosis, and trypanosomiasis to generate Holstein and Gir gene-edited lines. The project aimed to develop a high milk yield, heat tolerance, and disease resistance crossbreed dairy cow called “Slick Girolando,” which is a cross between Holstein and Gir edited lines. With its unique combination of traits, this innovative breed has the potential to revolutionize dairy production in SSA. The current study used a simulation analysis to determine the optimum breeding strategies for sire lines for 20 generations to enhance economically important traits in SSA. The traits considered were milk yield, disease resistance, survival rate, reproductive performance, heat tolerance, and longevity, which were selected based on African farmer breeding objectives. Four breeding strategies were evaluated: (1) crisscrossing Holstein-edited with Gir-edited sires, (2) crisscrossing Gir-edited with Holstein-edited sires, (3) modified crisscrossing strategies for Holstein-edited sires, and (4) modified crisscrossing strategies for Gir-edited sires. Variance components were estimated with the lme4 package, breeding schemes were plotted with the ggplot2 package, and sensitivity analysis was evaluated with the R 4.4.2 software. The sensitivity analysis identified that selecting a breeding strategy based on milk production was critical for the long-term success of dairy production in SSA. The modified crisscrossing strategy for Holstein-edited sire (using Holstein-edited line sires for 2 generations followed by Gir-edited line sires for one generation) maximized milk yield, improved other traits, and reduced inbreeding over 20 generations. This study offered valuable insights into breeding

strategies that can significantly improve milk production and overall genetic gain in African dairy production systems.

Key Words: crossbreeding strategies, gene editing, heat tolerance

1314 Correcting traditional reliabilities for unequal use of bulls in the genomic era. J. M. Tabet¹, I. Aguilar³, M. Bermann¹, D. L. Lourenco¹, I. Misztal¹, P. M. VanRaden⁴, Z. G. Vitezica⁵, and A. Legarra^{*1,2}, ¹University of Georgia, Athens, GA, ²Council on Dairy Cattle Breeding, Bowie, MD, ³Instituto Nacional de Investigacion Agropecuaria, Montevideo, Uruguay, ⁴Animal Genomics and Improvement Laboratory, Agricultural Research Service, United States Department of Agriculture, Beltsville, MD, ⁵INRAE, Toulouse, France.

Bulls with large numbers of daughters but only in a few herds had published reliabilities that were too high. Having many daughters in a few herds may lead to preferential treatment and cause biases. Fortunately, the US dairy cattle genetic evaluation always had before and has now a herd-sire interaction effect to model this possible bias. For bulls with daughters, genomic reliabilities combine purely genomic information with amount of information from daughters, which needs to be correctly quantified. Uneven distributions of daughters across herds happened in the past when natural mating was popular, then reduced with widespread use of artificial insemination, and is becoming more present with large use of very young genomic bulls in few, large herds. However, because reliabilities use approximations due to computational difficulties, the herd-sire effect may not be correctly accounted for in the approximated traditional reliabilities, which may result in overestimation of reliabilities for “odd” bulls with many daughters in few herds. We measured the possible overestimation in both simulated (“odd” sires had ~500 daughters in 1–2 herds, $h^2 = 0.20$; herd-sire variance ratio 0.05) and real data (milk yield for a reduced Jersey dataset; “odd” sires were those with an h-index of less than 6, where a sire with an h-index of n has at least n daughters in n herds, and “normal” bulls have an h-index >9 ; e.g., at least 10 daughters in each of at least 10 herds). We compared against the Misztal-Wiggans 1989 method (which does consider herd-sire) and further proposed a method to account for herd-sire in the Tier-Meyer 2004 approximation method for traditional reliabilities, and our idea reduces the information contributed from a cow to her sire, according to the size of the corresponding herd-sire. Exact average traditional reliabilities of “odd” bulls were 0.51 and 0.70 for simulated and real data. Ignoring the herd-sire effect led to optimistic values of 0.95 and 0.76. Modeling herd-sire led to more correct values of 0.50 and 0.65 (Misztal-Wiggans) or 0.58 and 0.66 (Tier-Meyer). We have tools to correctly model unequal repartition of genomic bulls across herds.

Key Words: reliabilities, herd-sire

1315 Approximating GEBV from observed to liability scales for health traits in dairy cattle. D. A. de Oliveira Padilha^{*1}, D. Lourenco¹, F. Bussiman¹, I. Misztal¹, D. Gonzalez², G. Vargas², G. de Oliveira², M. Sanchez², N. Vukasinovic², and J. Hidalgo¹, ¹Department of Animal and Dairy Science, University of Georgia, Athens, GA, ²Zoetis Genetics and Precision Animal Health, Kalamazoo, MI.

Genomic estimated breeding values (GEBV) for health traits are published as probabilities. To obtain GEBV as probabilities, the genetic analyses of these traits involve threshold models, yielding solutions on the liability scale. Although these models offer precision, they are computationally intensive and may encounter convergence issues. Linear models are a good alternative; however, solutions in the observed scale need to be approximated to the liability scale before converting

GEBV to probabilities. Previously, we derived an approximation from the observed to the liability scale with limited success for traits with extreme prevalence ($<5\%$). Our objective was to compare a new derivation with the previous one using health traits with very low ($<1\%$) to moderate prevalences (up to 25%). Data included up to 800K binary records for lameness, mastitis, retained placenta, ketosis, metritis, and displaced abomasum from multiparous Jerseys. Genotypes with 45K SNP were available for 200K animals. Univariate threshold and linear models were used to predict GEBV using software from the BLUPF90 family. The 2 approximations involved scaling GEBV in the observed scale: (1) the square root of the product of residual variance and the proportion of unexplained variance in the linear model, and (2) the square root of the product of the ratio of heritability in the liability scale to heritability in the observed scale and the phenotypic variance in the linear model. GEBV concordance from threshold and linear models in probability scale was assessed using Spearman correlation, regression parameters, and the overlapping of their distributions. No difference was observed in the correlations between GEBV from threshold and linear models across approximations, which ranged from 0.71 (very low) to 0.99 (moderate) but the regression parameters and overlapping of distributions were improved using the second (new) approximation. For traits with prevalence $>1\%$, the new approximation provides greater robustness and precision, making it a viable alternative for large-scale linear model analyses of categorical traits.

Key Words: binary traits, probability scale, threshold model

1316 Variance components estimation for complex models and very large datasets. M. Bermann^{*1}, A. Alvarez-Múnera¹, A. Legarra², I. Misztal¹, and D. Lourenco¹, ¹University of Georgia, Athens, GA, ²Council on Dairy Cattle Breeding (CDCB), Bowie, MD.

Likelihood inferences such as heritability estimation with REML or hypothesis testing are widely used in animal breeding and genetics. Due to memory usage and extremely long computational times, performing likelihood inferences is impossible for large datasets, especially with single-step GBLUP models (ssGBLUP). The main computational bottlenecks of likelihood inferences are the calculation of the log-determinant and the trace of a matrix product. This study aimed to derive and present methods to approximate the log-determinant and trace of the matrix product when genomic information is used, hence, making likelihood inferences for very large datasets possible. We proposed to approximate the log-determinant and trace using Monte Carlo sampling. For the log-determinant, this implied using notions of complex calculus and contour integrals. For the trace, the approximation involved the data simulation following ssGBLUP assumptions and fitting a surrogate linear model in a procedure known as Monte Carlo REML. We tested our proposed approximations using chicken, pig, beef, and dairy cattle datasets. The approximation of the log-determinant was 2 to 500 times faster than its exact calculation. The timing difference between exact and approximated log-determinant increased with the size of the data. For example, for the largest dataset, the exact calculation took 19 d, whereas its approximation took 1 h. The average absolute difference between the approximated and the exact value was 10^{-3} . We used the beef dataset to assess how the numerical approximations affect the likelihood calculation and heritability estimation. The exact and approximated log-likelihood showed maximums at the same heritability value. The approximated likelihood was used as a convergence criterion for Monte Carlo REML, making the iterations stop earlier without significant changes in the estimates. Our proposed approaches lifted the computing time and memory limitations for likelihood inferences in large datasets.

Therefore, approximated frequentist inferences are now possible for virtually any animal breeding dataset.

Key Words: variance components estimation, likelihood, single-step

1317 Large-scale multibreed single-step GWAS in US dairy cattle. E. R. Carrara^{*1}, M. Bermann¹, A. Legarra^{1,2}, I. Misztal¹, and D. Lourenco¹, ¹University of Georgia, Athens, GA, ²Council on Dairy Cattle Breeding, Bowie, MD.

Genome-wide association studies (GWAS) play a key role in identifying genetic variants linked to complex traits. However, traditional GWAS methods struggle to incorporate valuable information from non-genotyped animals. Single-step GWAS (ssGWAS) addresses this limitation by combining data from both genotyped and non-genotyped animals, making use of all available information. The statistical significance of markers can be determined by calculating the variance of their effects, which requires the inverse of the genomic relationship matrix (G^{-1}) and the prediction error (co)variance (PEC) of breeding values for genotyped animals. This process involves inverting the left-hand side (LHS) of the mixed model equations, which results in a dense matrix. For large-scale genotyped populations, ssGWAS can be efficiently performed using the algorithm for proven and young (APY), which calculates G^{-1} by splitting genotyped animals into core and noncore groups. In this approach, PEC is estimated from core animals instead of from the entire genotyped population. However, this method still requires the inversion of the LHS. To overcome this challenge, a method was developed to approximate PEC, which was previously validated against the exact method in a population of up to 450,000 genotyped animals. In this study, we applied this method to a much larger dataset, including about 100 million phenotypic records for 12 productive and reproductive traits (yield, fertility, and longevity group of traits). The analyses included about 53 million animals in the pedigree and about 2.4 million genotyped animals from the official US multibreed genomic evaluation. Despite the scale of the dataset, the method remained feasible, identifying genomic regions consistent with previously reported peaks for those traits (e.g., the *DGATI* for yield traits). The large volume of data strengthened the detected signals. These findings demonstrate the flexibility of ssGWAS with P -values obtained through approximate PEC, confirming its effectiveness for association studies in large-scale genotypic datasets. By integrating all available data, this approach enhances the potential for genomic discoveries and contributes to the understanding of the genetic architecture of important traits.

Key Words: genomic, P -value, SNP markers

1318 Algorithm for proven and young: Are core-dependent changes important? F. Bussiman^{*1}, J. Hidalgo¹, A. Cesarani^{1,2}, M. Bermann¹, D. Lourenco¹, and I. Misztal¹, ¹Department of Animal and Dairy Science, University of Georgia, Athens, GA, ²Departimento di Agraria, Università di Sassari, 07100 Sassari, Italy.

Single-step GBLUP (ssGBLUP) requires inverse of the genomic relationship matrix (GRM), which is computationally feasible with an algorithm for proven and young (APY). With APY, computing cost is cubic with the number of core animals and near-linear with the number of noncore. In various analyzes, different core sets cause fluctuations genomic estimated breeding values (GEBV) without affecting prediction accuracy. We aimed to investigate if these fluctuations are affected by limited marker numbers, genotyping errors, and core sizes. We simulated a selected population ($N_e = 100$, $h^2 = 0.25$, 1,000 QTL, 29 chromosomes) for 15 generations. Two marker densities were simulated: 70k or 135k.

The ideal number of core animals (core100%) was calculated as the number of eigenvalues explaining 99% of the variance in the GRM. Two additional core sizes were tested: half of the ideal (core50%) and double the ideal (core200%). Each core size was randomly sampled 3 times. Predictions were computed for each core and each sample, and the change was computed within sample for each size with respect to the true breeding value (TBV). To assess the impact of a limited number of markers, the 70k was randomly reduced to 35k. To evaluate genotyping errors, missing markers were set to the mean allele frequency. Finally, we tested all scenarios in terms of combinations between densities and core and noncore sets. In respect to the TBV, the average and maximum changes were constant regardless of the core size. Maximum changes increased 2-fold with limited or wrongly imputed markers or having core sets with less-quality genotypes. Maximum changes reduced by 10% when using 135k markers. Results were the same for limited number of markers or wrongly imputed. Having core animals in high density did not prevent fluctuations. The amount of change is equivalent to different blending strategies applied to the GMR. Although different core sets can cause GEBV fluctuations, these are inconsequential for selection. Limited marker density and genotyping errors exacerbate fluctuations, but don't affect accuracy. APY remains unbiased and robust for genomic predictions.

Key Words: algorithm for proven and young (APY), changes in genomic prediction, single-step GBLUP (ssGBLUP)

1319 Obtaining reliabilities of indirect predictions from SNP effects in single-breed and multibreed ssGBLUP evaluations. J. M. Tabet^{*1}, F. Bussiman¹, J. Hidalgo¹, A. Cesarani^{1,2}, I. Misztal¹, and D. Lourenco¹, ¹University of Georgia, Athens, GA, ²University of Sassari, Sassari, Italy.

Reliability (REL) assesses the quality of EBV. True reliabilities require the prediction error variance (PEV) from the inverse of the mixed model equations (MME), a computationally intensive process for large populations, so approximations are often used. When individuals have indirect predictions (IP) based on SNP effects, they are not included in the MME, making traditional methods to calculate their reliabilities unsuitable. We aimed to approximate IP reliabilities when SNP effects are calculated in large, genotyped populations. We compared 2 reliability approximations: (1) *RELIP*, based on the approximated prediction error covariance (PEC) of SNP effects based on core animals back solved from the genomic estimated breeding values, using single-step GBLUP (ssGBLUP) with the algorithm for proven and young (APY), and (2) using benchmark reliability, *RELGS3*, by calculating PEV in a ssGBLUP model using a block sparse inversion of the genomic relationship matrix with APY and effective record contributions. Data from the Council on Dairy Cattle Breeding included 98.9 million milk yield (MY) records from multiple breeds, 49.6 million animals in the pedigree, and 1.88 million genotyped animals. Reliabilities were calculated in multibreed and single-breed evaluations. Validation excluded pedigree, phenotype, and progeny data for focal animals born between 2019 and 2021. The reliabilities of IP were compared with a benchmark analysis that included focal animals' genotypes. In the multibreed analysis slopes were 0.95 (males) and 0.91 (females) for HO, -0.14 (males) and 0.63 (females) for JE, and 1.11 (males) and 0.99 (females) for BS. Correlations between both reliabilities were 0.95 (males) and 0.94 (females) for HO, -0.25 (males) and 0.40 (females) for JE, and 0.98 (males) and 0.80 (females) for BS. In the single-breed analysis, correlations improved for JE (0.99 for both sexes) and BS (0.99 for both sexes). Mean reliabilities in the single-breed were more consistent than in multibreed evaluations, demonstrating the possibility of approximating reliabilities based on

PEC of SNP effects of core animals in single-breed evaluations. With multibreed models, dominant breeds affect reliabilities, indicating the need for a within-breed approximation of IP reliabilities.

Key Words: single-step GBLUP (ssGBLUP), reliabilities, prediction error variance (PEV)

1320 Comparison of single-cell whole-genome amplification and genotyping methods: Implications for preimplantation embryo genomic selection in dairy cattle. S. Yan, S. Yan, H. Cui, S. Zeng, and Y. Zhang*, *China Agricultural University, Beijing, China.*

Preimplantation embryo genomic selection (eGS) represents a significant advancement in accelerating genetic improvement by shifting individual selection to the embryo stage. However, due to the minuscule DNA content obtained from embryo biopsies, whole-genome amplification (WGA) becomes an indispensable step before genome-wide genotyping. Multiple displacement amplification (MDA) and multiple annealing and looping-based amplification cycles (MALBAC) are the commonly used WGA techniques. This study was designed to comprehensively evaluate the amplification efficiency of MDA and MALBAC and their impacts on genome-wide genotyping in cattle. The different number of cells including 3-, 6-, and 9-cell groups were compared. Multiple screening strategies including Illumina Bovine GGP 100K array (SNP-array), genotyping by target sequencing (GBTS) and whole-genome sequencing (WGS) were utilized. The results revealed that MDA exhibited superior performance compared with MALBAC in multiple aspects, including the length and concentration of amplification products, SNP call rate, genotype concordance, and amplification bias. Additionally, both WGA methods showed an improvement in performance as the initial cell number increased from 3-cells to 9-cells. GBTS demonstrated a higher call rate than SNP-array and WGS. GBTS and SNP-array achieved the higher genotyping concordance and the lowest allele dropout rates than WGS. Notably, WGS detected a significantly larger number of SNP variants compared with SNP-array and GBTS, highlighting its potential in aneuploidy detection. Overall, this study offers valuable technical insights for the development of embryo genomic selection strategies in cattle breeding, which could potentially accelerate genetic improve of dairy cattle.

Key Words: whole genome amplification, genotyping, embryo

1321 Impact of genotyping errors on indirect predictions. A. Cesarani^{*1,2}, F. de Oliveria Bussiman¹, I. Misztal¹, and D. Lourenco¹, ¹*Animal and Dairy Science, University of Georgia, Athens, GA*, ²*Dipartimento di Agraria, Università degli Studi di Sassari, Sassari, Italy.*

Genomic evaluations initially included genotypes only in a secondary step after computing EBV based on pedigree and phenotypes. The single-step genomic BLUP (ssGBLUP) enables GEBV estimation in a single round, even when only a portion of the animals in the pedigree are genotyped. When the number of genotyped animals is too large, which prevents inverting the genomic relationship matrix, the algorithm for proven and young can be used. Another approach to reducing the computational cost of large-scale genomic evaluation is to include only informative animals in the main ssGBLUP evaluation and use indirect predictions (IP) for the non-informative ones. Thus, if a genotyped animal lacks progeny or phenotypes, its GEBV can be indirectly predicted from SNP solutions obtained in the main ssGBLUP evaluation. Besides the SNP effects, IP rely on the animal's genotypes, so accurate genotyping is essential for reliable IP. This study aimed to evaluate

the impact of genotyping errors on IP. A population of 15 generations was simulated using QMSim2, with a trait heritability of 25%, and genotypes simulated starting from generation 11. In the genotype file, we applied the following error rates: 0%, 5%, 10%, and 20%. A full ssGBLUP model was run using pedigree, phenotypic, and genotypic data from generations 11 to 15 to establish the benchmark GEBV. Then, ssGBLUP with data from generations 11 to 14 (training) were performed to obtain SNP solutions for subsequent use in IP. The ssGBLUP runs for the estimation of SNP effects considered 0%, 5%, 10%, and 20% genotyping errors. Then, SNP effects were used for IP considering the genotype file for animals in generation 15 (validation) also had these error rates. Results showed that the correlation between GEBV and IP decreased as the error rate increased: correlations decreased by 4 to 9 points according to the scenario. The impact of genotyping errors was similar whether the errors were present in the training or validation. For example, using the correct file to obtain SNP solutions and the file with 10% errors for validation yielded the same results as using the 10% error file to obtain SNP solutions and the correct file for validation. This highlights the importance of high-quality genotyping for the successful implementation of IP, especially when dealing with large datasets where computational efficiency is important.

Key Words: indirect prediction, genomic selection, accurate prediction

1322 Optimizing markers used for Jersey genomic prediction: Leveraging F_{ST} approach and imputed high-density SNP data. S. Toghiani*, D. J. Null, and P. M. VanRaden, *USDA, Agricultural Research Service, Animal Genomics and Improvement Laboratory, Beltsville, MD.*

High-density SNP panels and sequence data have significantly advanced complex trait analysis; however, their impact on genomic prediction remains limited, partly due to the high dimensionality of association models. The fixation index (F_{ST}), which identifies selection signatures by measuring allele frequency differences among populations, provides a promising SNP prioritization strategy. This study applied an F_{ST} -based approach using Net Merit as the target phenotype, enabling SNP prioritization across multiple economically relevant traits rather than a single trait. The impact of incorporating prioritized SNPs based on F_{ST} scores from an imputed HD panel (561,879 markers) into the official SNP map (69,200 markers) was evaluated for genomic prediction in the Jersey breed. F_{ST} scores were calculated using imputed HD genotype data from 15,609 Jersey bulls born before 2017, each with Net Merit reliability exceeding 75%. Bulls were sorted by their Net Merit scores, and the top and bottom 5% were classified into 2 subpopulations. F_{ST} scores were then estimated for all HD SNPs. The top 1% of SNPs with the highest F_{ST} scores were selected as candidate markers for tracking QTL and integrated into the official SNP map, resulting in a total of 74,171 markers. Genomic estimated breeding values (GEBV) from the December 2020 database for bulls born between 2017 and 2020 were correlated with their corresponding GEBV from the December 2024 database for 9 traits: Net Merit, productive life, somatic cell score, daughter pregnancy rate, heifer conception rate, cow conception rate, cow livability, early first calving, and heifer livability. The analysis was restricted to bulls with genomic and pedigree reliability exceeding 75% for each trait. The updated SNP map, incorporating prioritized markers, improved GEBV correlations by a 1% to 5% increase for 6 traits compared with the official SNP map. These findings highlight the value of F_{ST} -based SNP prioritization in enhancing genomic prediction accuracy by integrating markers under selection pressure.

Key Words: genomic prediction, fixation index, high-density SNP

Joint ADSA-CSAS Breeding and Genetics Symposium: Dairy Welfare—Breeding and Management Strategies

1323 Breeding for improved dairy cattle welfare and resilience: Opportunities in the genomics and phenomics era. L. F. Brito*, *Purdue University, West Lafayette, IN.*

Dairy cattle welfare is a critical aspect of sustainable and ethical livestock production, with significant implications for productivity, dairy product quality, consumer perception, and regulatory compliance. Traditional breeding programs have historically prioritized milk production traits, sometimes at the expense of welfare-related characteristics such as disease resistance, longevity, and heat tolerance. However, advances in genomics and phenomics now offer unprecedented opportunities to integrate welfare traits into breeding programs more effectively. As animal welfare is a multifactorial concept based on biological functioning, natural behavior, and affective states, there is a need for large-scale phenotyping of multiple indicator variables on a longitudinal basis. Recent developments in precision phenotyping, facilitated by automated sensors, imaging technologies, and machine learning, provide detailed, real-time data on key welfare indicators. In general, welfare traits in dairy cattle populations are heritable and, therefore, can be improved through direct genetic selection. The integration of genomics and phenomics allows for the development of genomic selection indexes that incorporate welfare traits without compromising productive efficiency. Genetic correlations between welfare-related and production traits must be carefully managed to avoid antagonistic selection responses. Furthermore, leveraging big data from commercial herds and integrating multi-omics approaches can enhance the predictive accuracy of genomic selection models. Furthermore, accounting for genotype-by-environment interactions in breeding and management decisions is crucial for improving dairy cattle welfare. In this context, the primary objectives of this talk are to discuss opportunities for integrating large-scale phenotypic and genomic data to improve dairy cattle welfare. More specifically, I will present examples of novel welfare and resilience traits that can be derived from precision technologies, including automated milk feeders, milking robots, and wearable sensors, as well as the genetic background of dairy welfare traits and strategies for incorporating welfare and resilience traits into dairy cattle breeding programs.

1324 Improving welfare through inclusive innovation: The story of WELL-E. E. Vasseur^{*1,3} and A. Baniré Diallo^{2,3}, ¹*McGill University, Ste Anne de Bellevue, QC, Canada*, ²*UQAM, Montreal, QC, Canada*, ³*Research and Innovation Chair in Animal Welfare and Artificial Intelligence (WELL-E), Ste Anne de Bellevue, QC, Canada.*

The welfare of animals managed by humans is integrally tied to, and ultimately dependent on, the welfare of the humans doing the managing. Any sustainable and effective change is thus dependent on collaborative solutions that address the needs of both the animals and people involved. Our approach relies on the use of IoT, computer vision, and machine learning to improve our ability to detect and monitor changes in animal welfare and longevity earlier than possible with visual methods, and to generate predictions to aid on-farm decision-making, ensuring that resources and efforts are focused on the animals most likely to succeed in the long term. Grounded in industry partnerships, members of the dairy community sit on both the scientific and management committees, where they contribute to research orientations, leading to the co-creation of research projects and initiatives. This collaborative approach not only ensures the applicability and relevance of all research but allows for

continuous feedback between the research and industry environments, creating a true digital living laboratory. Launched in 2023, our team has been conducting pilot research on 2 farms and working directly with staff and management to co-develop and pilot new practices and techniques for the management of animal welfare, to be disseminated across Canada and beyond. We work toward major paradigm shifts in both research (changing the way animal behavior and complex states are understood through new analytic techniques) and practice (our research informing major changes in the Canadian Dairy Code of Practice), with the goal of providing practical, long-term solutions for the improvement of animal welfare, while simultaneously addressing real-world problems farmers face along the way. Our collaborative approach promotes the responsible integration of new technologies to the dairy industry and empowers producers to be at the forefront of positive welfare developments, ensuring their sustainability and reinforcing the importance of stakeholder participation in innovative scientific research.

Key Words: inclusive innovation, animal welfare

1325 Exploring the role of nutrient adequacy in dairy cow health and welfare. D. E. Rico*, *CRSAD, Deschambault, QC, Canada.*

It is well known that adequate nutrient balance is a key aspect of optimal production performance of animals; however, its role on behavior, wellbeing, and health of animals is less well understood. Indeed, nutrient requirements of animals are often established based on physiological state and productivity levels, but rarely considering the impact of increased needs due to social or environmental stress. Furthermore, critical periods of a cow's life, such as early lactation, are associated with increased morbidity and reproductive problems, both leading to increased rates of culling. Interestingly, although negative energy balance and ketosis were historically presented at the probable cause of some of these issues, current research suggests an underlying role of inflammation in periparturient health. For instance, a recent study determined that up to two-thirds of cows with clinical ketosis also suffered from endotoxemia, highlighting a potentially central role of inflammatory status in the health and productivity of cows in early lactation. Importantly, some nutrients with anti-inflammatory effects such as vitamin D3 and long-chain omega-3 fatty acids have been linked to overall health status and to the modulation of inflammation. Although these nutrients are already part of dairy cow diets, their supply may be insufficient for periods of stress, such as early lactation or during heat stress. Key questions remain in this regard, as specific nutrient requirements for periods of stress are currently not well defined in dairy cows. This presentation explores the role of nutrients with potential to modulate animal health and well-being, highlighting focus areas for future research.

Key Words: nutrient requirements, dairy cow, welfare

1326 Identifying a sustainable path forward when looking to the future of dairy farming. M. A. G. von Keyserlingk*, *Animal Welfare Program, Faculty of Land and Food Systems, The University of British Columbia, Vancouver, BC, Canada.*

The current animal welfare challenges facing the dairy industry must be looked at in the context of the sustainability as the issues are increasingly

complex and the solutions will not always be solved by a simple technical solution. Although we have addressed some of the “low hanging fruit,” such as preventing tail docking and using pain mitigation when disbudding calves, many management practices that are known to compromise animal welfare are still common on many farms, such as feeding calves insufficient amounts of milk, providing social housing for calves, lack of access to pasture, lameness and transition cow disease among others. The failure to provide cows access to pasture, early life killing of surplus calves and the immediate separation of the calf from the cow at birth are particularly contentious as they fail to resonate with societal values. Moreover, the growing tension between suggested practices that attempt to address mitigate the environmental foot print of dairy farming and animal welfare are gaining traction in public discussions. A global discussion on how to preserve dairy cattle health and welfare is urgently needed, sparking a debate on what constitutes the ideal dairy system that balances animal productivity, animal welfare and the values of all stakeholders including the citizens who the industry relies on to buy dairy products. In shaping future dairy systems, all stakeholders must be given a voice, from farmers and scientists to policymakers and community members, acting as citizens and consumers, as the industry strives for a sustainable model that is environmentally friendly, economically viable, and socially acceptable. Listening to society and integrating their insights into our day-to-day discussions on what it means to be a good dairy farmer is an important step toward ensuring the industry’s continued evolution such that practices on farms align with ethical and ecological goals.

1327 Herd Sustainability Index: Using milk recording data to evaluate dairy herd sustainability. J. St John*, M. Séguin, and D. Lefebvre, *Lactanet, Sainte-Anne-de-Bellevue, QC, Canada.*

Producing milk sustainably has been linked to dairy cow longevity (i.e., length of productive life), production measures, and animal health. The

Herd Sustainability Index can help evaluate and compare herd sustainability indicators in Canada using milk recording data. This index includes 10 parameters, focusing on 4 primary concepts: (1) longevity and culling, (2) production and feeding, (3) replacement heifers, and (4) health. Parameters used to evaluate cow longevity are (1) cows in third or greater lactation, (2) involuntary culling, and (3) cow mortality rate. Parameters used to evaluate production and feeding are: (1) MUN <5 or >18 mg N/dL (%), (2) management score for milk production (evaluation of genetic potential vs. actual ECM), and (3) Transition Cow Index assesses the transition period’s success (actual vs. expected yield in early lactation). Parameters used to evaluate replacement heifer management are: (1) calf mortality rate 0 to 24 h (%), and (2) age at first calving (months). Parameters used to evaluate health are (1) cows with high BHB, an indicator of ketosis status in the herd, and (2) cows with high SCC. A percentile rank (1–99) is assigned to each of these 10 parameters based on dairy farms’ data across Canada, allowing producers to see their rank for each parameter. The Herd Sustainability Index is calculated as the sum of the percentile rank for the 10 parameters, with equal weight, and herds are reclassified into percentile ranks based on the sum. The Herd Sustainability Index can help producers identify areas to improve and track their improvement over time. Additionally, management strategies from top-ranking farms (75–99 percentile) can be assessed to help identify potential best management strategies. For example, top-ranking farms have been linked to using foot baths and hoof trimming at least three time a year. Overall, the Herd Sustainability Index stems from indicators developed using routine milk recording data to assist producers in evaluating their herd sustainability and can help identify good management practices on-farm.

Key Words: herd sustainability, herd management, milk recording

Dairy Foods: Chemistry

1328 The MetaBó-Bainne project—Development of the pasture-based milk metabolome database as determined by ¹H-NMR. P. Rojas-Gómez¹, R. Pariyani¹, E. Roche², M. Dineen², L. Bateman¹, D. Lynch¹, A. R. Maguire¹, T. B. Murphy³, N. A. McCarthy¹, J. A. O'Mahony¹, and T. F. O'Callaghan^{*1}, ¹University College Cork, Cork, Ireland, ²Teagasc, Cork, Ireland, ³University College Dublin, Dublin, Ireland.

The purpose of the MetaBó-Bainne project is to characterize the milk metabolome from pasture based dairy farms and assess the factors affecting its composition such as cow diet, seasonality, genetics, and examine the influence the milk metabolome has on milks techno-functional properties, and potential as prediction biomarkers for traceability, methane emissions and milk processability. Milk samples were collected on a weekly basis throughout 2023 (n = 533), from 10 commercial farms bulk tanks and 3 commercial shop brands. All samples were subjected to semi-untargeted metabolomic analysis using ¹H-NMR and Chenomx software. Raw milk samples were also examined for general composition, somatic cells and techno-functional properties including, heat stability and gelation characteristics. In excess of 27,000 quantified datapoints have been collected across the season comprised of metabolites, macronutrient components and techno functional characteristics throughout lactation. A total of 40 metabolites from diverse chemical classes such as amino acids, carbohydrates, fatty acids, and organic acids have been quantified. Multivariate analysis of data to date has demonstrated an evolution of the milk metabolome as lactation progresses. On-going statistical analysis have highlighted significant differences in the metabolome across lactation and correlations between key metabolites and techno-functional traits such as gelation properties and heat stability, and their potential to be used as predictors of processability and as biomarkers of pasture derived dairy products have been identified. The milk metabolome is diverse and affected by a variety of intrinsic and extrinsic factors which subsequently affect the quality and techno functional properties of milk. The subsequent development of the MetaBó-Bainne Milk Metabolome database will serve as a valuable resource for future research.

Key Words: milk metabolomics, pasture, seasonality

1329 In vitro simulated infant gastrointestinal digestion of camel and bovine milk colostrum: Peptide identification, molecular simulation, and antioxidant properties. S. Maqsood* and A. Ashfaq, *United Arab Emirates University, Al Ain, United Arab Emirates.*

Bovine milk is widely used in the production of infant milk formula. Recently camel milk has also become the center of attraction for researchers to develop infant milk due to its various nutritional benefits and the absence of major allergen β-LG. This comparative study on bovine and camel colostrum digestion under in vitro simulated infant gastrointestinal digestion provides insights into the protein digestion pattern and availability of bioactive peptides. This study aimed to assess the antioxidant properties of peptides obtained after in vitro simulated infant gastrointestinal digestion of camel and bovine colostrum. The protein digestibility of these 2 colostrum samples after gastric (1 h) and gastrointestinal (1 h) phase was evaluated using the degree of hydrolysis assay and SDS-PAGE. Furthermore, peptides generated after the gastric and gastrointestinal phase were then identified using LC-MS/MS and its interaction was studied with PDB:2FLU (Kelch-Neh2 Complex) using molecular docking and in silico analysis. All the experiments were conducted in triplicates. To determine the significant differences

between the samples, ANOVA was used, followed by Tukey's post hoc test ($P < 0.05$). The results showed that the degree of hydrolysis increased significantly after digestion as compared with the undigested samples from 604.33 ± 15.89 to $17,333.44 \pm 192.45$ μg NH₃/g in camel colostrum and $1,317.66 \pm 76.37$ to $10,414.55 \pm 195.31$ μg NH₃/g in bovine colostrum. Also, the antioxidant properties evaluated through DPPH and ABTS radical scavenging activities showed a significant increase after gastrointestinal digestion. It was also observed that the selected peptides (camel colostrum: FPLAP and EGGFDAKLMFIV; and bovine colostrum: PGPPGTPGPI and SLVYPFPGPI) could form stable hydrophobic and hydrogen bond with Keap 1 protein, thereby potentially activating the Keap 1-Nrf2 signaling pathway. Overall, in this study, the digestibility and antioxidant properties of colostrum samples showed their potential in fortifying infant milk formula.

Key Words: colostrum, simulated infant digestion, peptide, antioxidant properties, camel milk, molecular docking

1330 Galactooligosaccharides from whey permeate streams. M. S. Mohan*, *South Dakota State University, Brookings, SD.*

Whey processing involves fractionation of whey protein and lactose from sweet whey, as the retentate and permeate streams, by filtration processes. The whey permeate streams are a cost-effective raw material for galactooligosaccharides (GOS) production, in place of the traditionally used refined lactose powder. Galactooligosaccharides have been gaining importance owing to its positive effects on gut health; however, application has been limited to infant formula, owing to the high cost of the GOS powders and syrups available in the market. We investigated the use of different enzymes to produce GOS from whey permeate streams (WPS). Our findings indicate that commercially available β-galactosidase enzymes can hydrolyze lactose into glucose and galactose and further initiate transgalactosylation to produce GOS, with up to 5 degrees of polymerization. The utilization of WPS with 5% lactose concentration, produces the maximum amount of GOS after 180 min at 40°C, at pH 6.8 and 4.5 for commercial β-galactosidase enzymes from yeast and fungus, respectively. The remaining unutilized glucose, galactose and lactose can also be effectively converted to GOS by certain yeast, mold, and bacteria with β-galactosidase enzyme activity. We identified certain strains of *Kluyveromyces marxianus* producing up to 2,000 Miller enzyme units/mL of β-galactosidase after 14 h of fermentation. There is potential for utilizing enzymes and microorganisms with β-galactosidase enzyme activity, in combination, for efficient GOS production from WPS. Hence, the utilization of WPS for GOS production can provide low-cost dietary fiber options for use in different food products and dietary supplements.

Key Words: lactose, lactase, dietary fiber

1332 Understanding the physical stability of whey permeate powders: Effect of composition and water relationships. D. Sözeri Atik*, N. Mirfakhraie, and J. Ubbink, *Department of Food Science and Nutrition, University of Minnesota-Twin Cities, Saint Paul, MN.*

Whey permeate powders exhibit several issues during transport and storage related to the physical instability of the powders. This research aimed to evaluate the composition and water relationships of whey permeate powder samples to understand their physical stability. For this purpose, 15 commercial whey permeate powder samples were collected

from 4 different producers in the United States and stored in mylar bags at -80°C , then subjected to the composition analysis. Analysis of variance and Tukey tests were conducted with a significance level of 0.05 to determine the statistical differences in composition among the samples. The lactose, glucose, and citric acid content of the samples significantly varied between $69.45\% \pm 3.24\%$ and $82.58\% \pm 4.35\%$, $0.10\% \pm 0.01\%$ and $0.19\% \pm 0.03\%$, and $0.42\% \pm 0.24\%$ and $0.83 \pm 0.07\%$ ($P < 0.05$), respectively. The critical variation was observed in the lactic acid and galactose content among the samples, which ranged from $0.10\% \pm 0.03\%$ to $2.97\% \pm 0.05\%$ and from $0.23\% \pm 0.06\%$ to $2.95\% \pm 0.04\%$ ($P < 0.05$), respectively. Interestingly, the 3 samples with the highest concentrations of lactic acid and galactose had the lowest glass transition temperature (T_g), which underlines the importance of these low molecular weight (LMW) components in the plasticization effect on the powder stability. Preliminary results indicate that powder browning correlates with the galactose and lactic content of the powders. In addition, the water vapor sorption isotherms of the samples were determined and the dependence of the T_g on the water content was modeled using the Gordon–Taylor equation. At a water activity (a_w) of 0.11, the T_g of 4 powder samples is below 15°C , and at $a_w = 0.23$, the T_g of 8 samples is below 10°C . Consequently, the amorphous phase of the permeate powders is in the high-mobility rubbery state at ambient conditions, indicating they are highly unstable. Overall, the physical stability of whey permeate powders is significantly affected by their composition and water content, as water and LMW compounds serve as plasticizers that reduce T_g , rendering powders highly susceptible to caking, stickiness, and chemical reactions.

Key Words: whey, permeate, lactose

1333 Exploring the prebiotic potential of whey protein phospholipid concentrate in promoting growth and binding of *Limosilactobacillus reuteri*. S. Mohapatra*, J. Oh, J. Rugji, J. P. P van Pijkeren, X. Pan, and G. Ozturk, *University of Wisconsin–Madison, Madison, WI*.

Whey protein phospholipid concentrate (WPPC) is a byproduct of whey protein isolate production and is underused within the dairy industry. The WPPC is rich in glycosylated bioactive compounds, including milk fat globule membrane (MFGM) proteins, which make up 23% of the total relative protein content. These glycoproteins, including mucins, offer potential binding properties to bacterial cell surface proteins and resources for probiotic growth. This study aims to evaluate the prebiotic potential of WPPC in enhancing the growth and binding affinity of the probiotic *Limosilactobacillus reuteri*. To assess this potential, we used the gut symbiont *Limosilactobacillus reuteri* ATCC PTA 6475, an established probiotic that binds mucus. First, we enriched the glycoproteins using sequential microfiltration and ultrafiltration, resulting in enriched glycoproteins free of simple sugars. Subsequently, we examined the binding capacity of these glycosylated proteins from the WPPC fraction to *L. reuteri*, as well as their ability to support in vitro *L. reuteri* growth. Our results indicate that, based on SDS-PAGE analysis, that glycosylated WPPC proteins and *L. reuteri* form a complex. Microscopy of crystal violet staining showed binding between WPPC and *L. reuteri*. Further evaluation of bacterial survival in modified MRS (mMRS) and PBS at 37°C and 4°C in the presence or absence of WPPC demonstrated enhanced viability (but they do not grow) with WPPC during the first 3 d. However, after 3 d, the rate by which microbial viability was reduced was comparable in samples with or without WPPC. Notably, mMRS supplemented with WPPC enhanced the growth of *L. reuteri*. Subsequent growth studies in 3 defined media consisting of all nutrients without (1) carbohydrates (LDMa), (2) amino acids (LDMb), and (3) fatty acids (LDMc) indicated that sugars released from WPPC promote

the growth of *L. reuteri*. In sum, our findings provide a foundation to further investigate the potential of WPPC as a prebiotic with potential applications in industry, agriculture, and health care.

Key Words: whey protein phospholipid concentrate (WPPC), prebiotic, *Limosilactobacillus reuteri*, WPPC-*L. reuteri* complex

1334 Bovine milk fat globules as a probiotic delivery vehicle and treasure trove for bioactives. K. Ambatipudi*, M. Yadav, and A. Kapoor, *Indian Institute of Technology Roorkee, Roorkee, Uttarakhand, India*.

The milk fat globule membrane (MFGM) and *Lactobacillus* modulate the infant's gut and benefit health with limited information on their positive effect on the intestine. Similarly, the MFGM lipids are essential for human nutrition and food production, yet their variation across industrially relevant animal species remains poorly understood. The current study assessed the probiotic potential of *Lactiplantibacillus plantarum* (MRK3), *Limosilactobacillus fermentum* (MK1) isolated from feces, and its interaction with MFGM during conditions mimicking the infant digestive tract, followed by MFGM lipidomic profiles by mass spectrometry from Holstein Friesian cows (HF, $n = 9$) and Murrah buffaloes (Mu, $n = 9$). Both strains showed high tolerance to gastrointestinal conditions, cell surface hydrophobicity, and strong anti-pathogen activity against *Staphylococcus aureus*. The MFGM significantly exhibited xanthine oxidase activity, membrane roughness, and surface topography during digestion. In the presence of MFGM, the survival of MRK3 was higher than MK1, and electron microscopic observation revealed successful entrapment of MRK3 in the membrane matrix throughout digestion. Interestingly, probiotic-membrane matrix interaction showed significant (1-way and 2-way ANOVA; $P < 0.05$) synergy to alleviate oxidative stress and damage induced by cell-free supernatant of *Escherichia coli* in Caco-2 cells. Mass spectrometry led to identification of 313 lipid species, including triacylglycerols ($n = 160$), diacylglycerols ($n = 15$), phosphatidylcholines ($n = 30$), phosphatidylethanolamines ($n = 16$), phosphatidylserines ($n = 5$), phosphatidylinositol ($n = 3$), lysophosphatidylcholines ($n = 3$), sphingomyelins ($n = 22$), and ceramides ($n = 1$). Lipid profile variations between the animals were analyzed by examining relative concentrations of individual lipid species and groups based on fatty acid saturation levels or chain lengths (tested using Student's *t*-test). The total phospholipid content was higher in HF (45.1%) than in Mu (38.4%). Eighteen differential lipid species (Fold change >2 or <0.5 ; $P \leq 0.05$; variable importance in projection score >1) significantly separated the samples between the animals were identified. These findings provide crucial insights into the probiotic delivery potential of MFG and biological variability of MFGM lipids across species, with implications for nutrition and dairy science.

Key Words: milk fat globule, probiotic delivery vehicle, membrane, lipid, mass spectrometry

1335 Adhesion of probiotic cells to the surface of milk fat globules. W. P. Withanage^{1,2}, D. W. Everett^{*1,3}, M. Waterland^{1,3}, and C. Thum^{1,2}, ¹Riddet Institute, Palmerston North, New Zealand, ²AgResearch, Palmerston North, New Zealand, ³Massey University, Palmerston North, New Zealand.

The milk fat globule (MFG) surface provides protection to probiotic cells during transit through the gastrointestinal tract to allow colonization in the gut and health benefits to a human host. The mechanisms by which cells adhere to the MFG membrane (MFGM) are not conclusively known. Eight probiotic strains—*Lactocaseibacillus rhamnosus* LGG,

Lactocaseibacillus rhamnosus HN001, *Bifidobacterium infantis* NLS, *Bifidobacterium breve*, *Lactocaseibacillus paracasei*, *Lactiplantibacillus plantarum* lp299v, *Bifidobacterium longum* 1714 serenitas, and *Saccharomyces boulardii*—were added to bovine MFG suspensions and the extent of adhesion determined by flow cytometry, with cells stained by acridine orange and MFGs by 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) ammonium salt. Cell surface hydrophobicity was measured by vortexing with hexadecane and optical density (OD) measurements at 600 nm of the aqueous phase after sedimentation. Values were $26.1\% \pm 1.2\%$, $11.96\% \pm 0.05\%$, $25.1\% \pm 1.7\%$, $22.9\% \pm 1.3\%$, $43.6\% \pm 0.8\%$, $37.0\% \pm 0.4\%$, $21.55\% \pm 0.12\%$ and $32.3\% \pm 1.3\%$ for the cultures in the order listed above. Zeta potentials were measured in 20 mM imidazole, 50 mM NaCl, 5 mM CaCl₂ pH 7 buffer, and were -2.4 ± 0.6 , -4.1 ± 0.5 , -11.4 ± 1.0 , -3.0 ± 0.3 , -2.9 ± 0.3 , -10.0 ± 0.6 , -10.4 ± 0.6 and -3.0 ± 0.6 mV for the cultures in the order above, and -10.8 ± 0.3 mV for MFGs. Cellular surface proteins were analyzed after extraction with 5 M LiCl, 12,000 × *g* centrifugation, 3.5 kDa cut-off dialysis against water, and SDS-PAGE. Growth kinetics of cultures were determined over 48 h by changes in OD at 600 nm in cream, butteroil, buttermilk, or a buttermilk and butteroil mixture for *B. infantis* and *L. rhamnosus* HN001 (3%–6% adhesion to MFGs) and *B. breve* and *L. rhamnosus* LGG (97%–99% adhesion to MFGs), and was highest in buttermilk for all 4 species, indicating the importance of MFGM components on growth. Flow cytometry background acceptance values for adhesion ranged from 0.11% to 0.46%. No clear trend was observed between adhesion and either hydrophobicity or zeta potential, suggesting a possible role for cell surface proteins in the binding of probiotic cells to MFG surfaces. These results show the potential for MFGM in the formulation of functional dairy products with enhanced probiotic activity.

Key Words: probiotic, milk fat globule membrane (MFGM)

1336 Impact of heat treatment and β -casein genotypes A1A1 and A2A2 on β -casomorphin-5 and -7 release during in vitro

gastrointestinal digestion. S. Serhan*, E. V. Moposita Lasso, W. Li, X. Such, G. Caja, and A. A. K. Salama, *Group of Ruminant Research (G2R), Department of Animal and Food Sciences, Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona, Spain.*

This study evaluated the effect of heat treatment (HT) and β -casein (CN) genotype on the levels of β -casomorphin-5 (BCM-5) and β -casomorphin-7 (BCM-7) before and after in vitro gastrointestinal digestion of milk. Milk samples were collected from cows genotyped for β -CN, κ -CN, and β -LG. Samples were grouped by genotype and pooled to form 3 composite samples: (1) A1*: A1A1 β -CN and a κ -CN genotype that includes at least one E variant, (2) A1: A1A1 β -CN and AB κ -CN genotype, and (3) A2: A2A2 β -CN and AB κ -CN genotype. The β -LG genotypes were evenly distributed in A1 and A2 (50% AA, 50% AB). Pools were skimmed, heat-processed (63°C, 30 min), and subjected to in vitro digestion, including gastric (G120) and intestinal (I120) phases for 120 min. The ELISA was used for the detection of BCM-5/7. Data were analyzed using PROC MIXED in SAS (ver. 9.4). The model included the effects of genotype, heat treatment (raw and HT), digestion phase (milk, G120, and I120), and their interactions. The BCM-7 concentrations in I120 exceeded the detection range of the ELISA kit and were not included in the results. The BCM-7 levels were highest ($P < 0.05$) in A1* raw milk (30.97 ± 4.19 ng/mL), followed by A1 (16.80 ± 4.19 ng/mL), and lowest in A2 (1.32 ± 4.19 ng/mL). The BCM-7 was released across all genotypes after digestion of raw milk, with the lowest levels ($P < 0.0001$) in A2 G120 milk (5.72 ± 4.19 ng/mL). Digestion of HT milk resulted in a lower BCM-7 increment in A1* (+89%; $P < 0.01$) and even reduced BCM-7 levels for A1 (−46%; $P < 0.01$) and A2 milk (−54%; $P = 0.87$) compared with unheated milk. The BCM-5 levels were higher ($P < 0.01$) in A1 (6.12 ± 0.49 ng/mL) compared with A1* and A2 (3.87 and 2.28 ± 0.47 ng/mL, respectively). In conclusion, HT reduced BCM-7 release after digestion across all genotypes but had no effect on BCM-5. Additionally, BCM-7 and BCM-5 levels were lowest in A2 milk before and after digestion compared with A1 milks.

Key Words: β -casein, β -casomorphin-5, β -casomorphin-7

Dairy Foods Symposium: Health Benefits of Cheese

1337 The health benefits of cheese. K. Ricklefs-Johnson*, *National Dairy Council.*

Cheese, a dietary staple across various cultures, offers numerous health benefits that extend beyond its rich and diverse flavors. Historically, cheese has been valued as a concentrated form of milk with the added advantage of an extended shelf life. It served earlier generations as an energy-dense and nutritious food source. Despite the higher levels of sodium and saturated fat compared with other dairy products, a growing body of evidence suggests that cheese consumption may be linked to a reduced risk of type 2 diabetes (T2D) and a lower risk of stroke. This discrepancy between the expected and observed health impacts of cheese may be attributed to the unique physical structure and composition of proteins, vitamins, and minerals in cheese, known as the dairy matrix. Moreover, cheese is a rich source of essential nutrients, including high-quality protein, bioactive peptides, amino acids, fats, fatty acids, and vitamins A, D, and B12, as well as minerals. The contributions of these nutrients to bone health, muscle function, and cardiovascular wellness are well established. Specifically, calcium and vitamin D are crucial for maintaining bone density and preventing osteoporosis. The protein content in cheese supports muscle repair and growth, while dairy's unique fatty acids and bioactive peptides may help reduce inflammation and regulate blood pressure. The goal of this presentation is to examine recent scientific research that continues to substantiate the value of cheese consumption as part of a healthy diet, discuss the potential health benefits of cheese consumption in relation to chronic diseases and explore the concept of the dairy matrix and how the unique composition of proteins, vitamins, and minerals in cheese may contribute to its health benefits.

Key Words: cheese, dairy foods, cardiometabolic

1338 Cheese as a unique matrix: Bioaccessibility and bioavailability of nutrients. S. Zhou^{*1}, A. Brodtkorb², J. J. Sheehan², E. R. Gibney¹, and E. L. Feeney¹, ¹*University College Dublin, Institute of Food and Health, Dublin, Ireland,* ²*Teagasc Food Research Centre, Fermoy, Ireland.*

Epidemiological evidence now supports that cheese may be beneficial to cardiovascular health. Cheese is a particular example of the dairy food matrix, containing proteins and bioactive compounds that may benefit cardiovascular health despite its high saturated fat content. A key emerging factor is vitamin K (VK), which is involved in blood coagulation and bone and cardiovascular health. Two main forms exist: phyloquinone (K1) from plants and menaquinones (K2, MK4–13) from bacterial fermentation. A source of VK, and especially K2, cheese can vary considerably in VK across different varieties. This is due to multiple factors, including milk source, fermentation processes, and starter cultures. Bovine feeding can affect K1, and we previously reported significantly greater vitamin K1 in Irish Cheddar cheese derived from pasture feeding, vs. Cheddar derived from Total Mixed Ration (TMR) feeding, from a controlled animal feeding trial. It is unknown whether cheese from different feeding systems can result in differences in human VK status. This study investigated the bioavailability and bioaccessibility of vitamin K in pasture-derived and TMR-derived Cheddar cheese. Plasma and serum samples from a 6-wk human intervention study (120 g/d pasture-fed Cheddar cheese [GF-CC; n = 33] vs. TMR-fed Cheddar cheese [TMR-CC; n = 27]) were analyzed for markers of vitamin K status: dephosphorylated, uncarboxylated matrix Gla protein (dp-ucMGP; MyBioSource), undercarboxylated osteocalcin (ucOC), and

carboxylated osteocalcin (cOC), via competitive enzyme immunoassay (Takarabio, France). Vitamin K bioaccessibility was investigated using an in vitro digestion model (INFOGEST 2.0). Total and individual VK vitamers (VK1, MK-4, MK-7, MK-9) were analyzed using HPLC-FLD (Agilent Technologies). The TMR-CC group had greater total VK and MK-9, whereas GF-CC had higher K1 and MK-4 versus TMR-CC (both $P < 0.05$). Despite these differences, results indicated similar improvements in VK status in both intervention groups after consumption. Follow-up in vitro digestion studies of the cheeses from different feeding systems suggest greater bioaccessibility of K1 and MK-4 from GF-CC ($P < 0.05$), which may have contributed to these findings.

Key Words: cheese, vitamin K, bioavailability

1339 Enhancing the bioactive properties of cheese. R. A. Ibáñez*, *Center for Dairy Research, University of Wisconsin–Madison, Madison, WI.*

Due to combined action of native milk enzymes, addition of rennet, and microbial activity from starter and nonstarter lactic acid bacteria, intact caseins (CN) are hydrolyzed into peptides and free amino acids. In recent years, the occurrence of low-molecular weight peptides presents in cheese (i.e., 2–20 amino acid residues) has brought the attention of dairy food scientists from both academia and industry. These peptides have the potential to exhibit physiological responses in human metabolism, which may include antimicrobial, antihypertensive, immunomodulatory, antioxidant, opioid and other activities. These compounds, named bioactive peptides (BP), typically accumulate during cheese ripening, and may require extended time to achieve a significant biological activity. Release is strongly affected by a combination of several factors, including genetic variants of CN, cheese manufacture protocols, ripening conditions and extent of proteolysis. The fate of BP in cheese can also be influenced by gastrointestinal digestion after consumption and must be bioavailable to exhibit a physiological response. Several approaches aimed at improving the bioactive properties of cheese have been proposed, which typically rely on modulating cheese proteolysis (e.g., accelerated ripening). Modifications to typical manufacturing approaches have also been proposed as alternative methods to increase the bioactive properties of cheese. Therefore, cheese is a food product that can be used as a carrier of natural compounds that can positively impact upon human health.

Key Words: cheese, proteolysis, bioactive peptides

1340 Incorporation of health and wellness ingredients in cheese: Challenges and opportunities. J. J. Jaeggi*, *Center for Dairy Research, University of Wisconsin–Madison, Madison, WI.*

Consumers in the United States enjoy cheese in many ways. Whether as part of a food system or eaten plain, consumption has increased over the past several years. Cheese is a reliable source of essential nutrients such as calcium, phosphorus, protein, vitamin A, vitamin B12, and zinc. Some cheese may contain probiotic cultures and bioactive peptides, a derivative of protein hydrolysis in cheese. Cheese manufacturing techniques or even varieties can be made to reduce fat, sodium, or carbohydrate intake in addition to optimizing protein and calcium contents. Cheese provides the perfect vehicle in which to incorporate various ingredients to augment health and wellness. Adding nutraceuticals to cheese during the manufacturing process has been tested and, in some instances, fully

commercialized. Use of prebiotics such as inulin or other plant-based fiber can help increase satiety by limiting caloric intake. The addition of plant-based phytosterols, n-3 fatty acids, antioxidants, and many other options are possible. However, adding ingredients to cheese can result in a multitude of challenges, with ingredient cost being among the biggest. Ingredients can come in many shapes and sizes including particulates, powders, water, or oil soluble materials. Ingredients are typically added to milk fat, milk, or cheese curd before salting or hooping. Manufacturing considerations such as ingredient loss into the whey resulting in processing issues, allergen concerns, or cross contamina-

tion of equipment or brines are a concern for large-scale production or conversion facilities. However slight modifications in manufacturing techniques or facilities designed specifically for the manufacture of these nutraceutical cheeses can be successfully used to reduce ingredient costs and minimize manufacturing losses. Historically, consumers have not been willing to pay the added cost per kilogram needed to manufacture cheeses with health and wellness ingredients, but with the aging of our society, there is a chance the timing may be favorable.

Key Words: cheese, health, ingredients

Lactation Biology 2: Mammary Health

1341 Factors involved in the response to intramammary pressure by air inflation of the udder. E. M. Shangraw and T. B. McFadden*, *University of Missouri, Columbia, MO.*

Raising intramammary pressure (IMP) to physiological limits by air inflation of the udder reduces milk yields. Our objectives were to determine how the mammary gland responds to an initial elevated IMP and which factors explain this reduction. Ipsilateral glands of Holstein cows ($n = 8$) were randomly assigned to 40 mmHg air inflation of both glands (TRT) immediately after a.m. milking; contralateral glands were not inflated (CON). After treatment, glands remained inflated for 12 h, then cows were remilked. All glands were milked every 12 h. We collected milk samples per gland from bulk milk and strip milk samples taken at 0, 4, 8, 12, and 24 h after inflation. Mammary blood flow (MBF) through both pudendoepigastric trunks was measured by transrectal color Doppler ultrasound immediately before milking and at 4, 8, 12, and 24 h after inflation. Arteriovenous blood samples were collected from the tail and both milk veins <15 min before and after inflation, then before milk sampling. Milk and blood data were analyzed by 2-way ANOVA with repeated measures, wherein quarter within cow or cow was the subject, respectively. Non-normal data for IMP and mammary uptake were analyzed by *t*-tests. All TRT glands achieved target IMP but dropped $56\% \pm 5\%$ by 4 h. Milk yields in TRT glands were 66.6% and 39.6% lower than CON glands at 12 and 24 h, respectively ($P < 0.01$). Yields of fat, total protein, and lactose decreased with milk yield. Percentages of fat and protein in milk increased in TRT compared with CON glands at 12 h ($P < 0.05$) but milk lactose percent decreased ($P < 0.001$). At 12 h, SCC was markedly higher in TRT than CON glands and remained higher at 24 h ($P < 0.001$). For MBF, flow velocity (FV) decreased in CON but not TRT glands at 4 h. Then at 8 h, FV increased in CON but decreased in TRT glands. By 12 h, FV was not different between treatments. Mammary uptake of glucose and calcium was not different between treatments. Plasma lactose in TRT milk veins increased markedly within 15 min of inflation. Arterial, and CON venous, plasma lactose concentrations were not different from TRT by 4 h. Plasma lactose from all sites remained higher than pre-inflation ($P < 0.001$) until 24 h, when no longer different. Reduced milk yield after air inflation was partially explained by leakage of lactose but not by reduced extraction of nutrients.

Key Words: milk stasis, insufflation, nutritive blood flow

1342 Effect of intramammary infusion of chitosan on immune cell infiltration and mammary health in dairy cows. J. Gao*, Y. B. Amancio, N. Andriotti, C. G. Savegnago, T. N. Marins, A. M. Roper, and S. Tao, *Department of Animal and Dairy Science, University of Georgia, Athens, GA.*

Chitosan has chemoattractant and immune-stimulating effects. We aimed to evaluate the impact of intramammary infusion of chitosan at dry-off (DO) on immune cell infiltration and mammary health in Holstein dairy cows. In experiment 1, 6 healthy lactating cows with quarter milk SCC <200,000 cells/mL and no common mastitis-causing bacteria were enrolled. Quarters within each cow were randomly infused (100 mL/quarter) with one of 3 chitosan solutions (containing 125, 62.5, or 31.25 mg of chitosan), or a placebo (PL, 0.25% [vol/vol] acetic acid solution). Quarter milk samples were collected every 12 h after infusion to assess SCC. Intramammary infusion of 125 mg of chitosan resulted in the most significant increase ($P < 0.01$) in milk SCC at 12 h postinfusion

and was therefore selected for the follow-up study. In experiment 2, 46 healthy cows without mastitis in the last 3 mo of lactation and with milk SCC <200,000 cells/mL before DO were randomly assigned to (1) PL ($n = 22$): each functional quarter was infused with 50 mL PL after last milking at DO; (2) CH ($n = 24$): each functional quarter was infused with 50 mL of solution containing 125 mg of chitosan after last milking at DO. No intramammary antibiotics were applied, and an external teat dip was used to facilitate milk sample collection. Composite milk samples (by cow) were collected on -14, -7, 0, 1, 2, 4, 7 d relative to DO (DRD) to assess SCC, immune cell differentiation, and bovine serum albumin (BSA). Blood hematological profiles were assessed on 0, 1 and 7 DRD. Mastitis incidence from DO to 3 d after calving was recorded. Data were analyzed using PROC MIXED and LOGISTIC of SAS. Mammary secretions from CH cows had greater ($P \leq 0.05$) concentrations of SCC, neutrophils, lymphocytes, macrophages, and BSA in the early dry period than PL. On 1 DRD, CH cows tended to have lower circulating neutrophils than PL ($P = 0.07$). From DO to 3 d after calving, quarters infused with CH tended ($P = 0.08$) to have a lower incidence of clinical mastitis than PL (6.74% vs. 15.29%). In conclusion, intramammary infusion of chitosan at DO enhances immune cell infiltration and improves mammary health.

Key Words: chitosan, dry-off, immune cells, mastitis

1343 Udder health and short- and long-term milk yield in Holstein cows with delayed calf removal. A. D. Beard*, G. Frederick, R. Ewing, M. Wieland, and S. Mann, *Cornell University College of Veterinary Medicine, Ithaca, NY.*

Standard dairy practice is to separate cow and calf within a few hours of birth to promote cow and calf health and maximize milk harvest. The objective of this study was to determine the effects of delayed calf removal (DCR) on intramammary infection (IMI) and early lactation milk yield. Primiparous ($n = 21$) and multiparous ($n = 31$) Holstein cows were blocked and randomized to immediate calf removal (ICR; $n = 35$) or DCR at 5 DIM (DCR; $n = 17$). Individual cow-calf pairs were housed in the same conditions. All cows were milked 2×/d until 7 DIM and 3×/d from 8 DIM to wk 9. On d 5, DCR calves were removed before milking and were not reunited. On d 0 and d 7 after milking, quarter-level milk samples were collected aseptically and submitted for bacterial culture. Immediately after milking on d 3 and d 7, 20 USP units of intravenous oxytocin were given, then milking unit was reattached within 60 s to measure residual milk yield. Daily milk yield was composed of consecutive evening and morning milk weights; d 0 and d 1 were removed for analysis. Milk yield was analyzed using repeated measures ANOVA, with fixed effects of treatment (trt), day, trt × day, and parity (JMP Pro 17), and results reported as LSM ± SE. New IMI (NIMI) were analyzed with quarter as unit of analysis nested within cow within block (PROC GLIMMIX of SAS). Total milk yield of DCR from d 3 to d 5 was 29.5 ± 1.9 kg lower than ICR ($P < 0.01$). Daily milk yield was not affected by trt ($P = 0.92$) on d 6 and d 7 ($P = 1.0$), and from wk 2 (33.2 ± 1.0 kg) to wk 9 (43.2 ± 1.0 kg). There was no trt difference in residual milk on d 3 or d 7 ($P \geq 0.94$). No differences in the odds for NIMI by trt or parity were present ($P \geq 0.53$). Fisher's exact test revealed no trt difference for the proportion of chronic IMI ($P = 0.22$). Calf removal at 5 DIM did not alter the risk for new or chronic IMI, reduced milk yield exclusively during calf-contact, and was not a result of disrupted milk letdown. Milk yield rebounded in the first 24 h after calf removal and persisted through the first 9 wk of lactation.

Overall, udder health and long-term milk yield were not compromised by delaying calf removal to d 5.

Key Words: cow-calf contact, intramammary infection, milk yield

1344 Blackberry pomace-derived phenolic compounds reduce oxidative stress in bovine mammary epithelial cells. A. Griffin^{*1}, H. Eldaous^{1,2}, S. Rajput¹, C. O. Lemley¹, X. Zhang¹, and J. Halfen¹, ¹Mississippi State University, Mississippi State, MS, ²Benha University, Mushtuhur, Toukh, Qalioubia, Egypt.

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

This study evaluated blackberry pomace-derived phenolic compounds (PC) as natural antioxidant compound in bovine mammary epithelial cells (MAC-T) challenged with hydrogen peroxide (H₂O₂). PC were extracted from dried, ground blackberries, and their total antioxidant capacity was measured using the Folin–Ciocalteu method (expressed as mg of gallic acid equivalents [GAE]/g dry weight [d.w.]). MAC-T cells, cultured in 75 cm² flasks to 85% confluency, were plated at 100,000 cells/mL in a black 96-well plate, and incubated for 24 h at 37°C with 5% CO₂. Cells were preincubated with or without PC for 12 h, and then treated with or without 600 µM H₂O₂ for 6 h. Treatments included control (media only), H₂O₂ (600 µM), 50 mg/mL PC (no H₂O₂), 50 mg/mL PC (600 µM H₂O₂), 100 mg/mL PC (no H₂O₂), and 100 mg/mL PC (600 µM H₂O₂). Oxidative stress (OS) was quantified using an intracellular reactive oxygen species (ROS) assay kit (BioVision Inc.). Data were analyzed using the MIXED procedure of SAS, including a total of 4 replicates, with ROS expressed as fold change over the control. Antioxidant capacity of PC treatments was 267.93 mg GAE/g d.w ± 7.7 and 288.76 ± 3.0, for 50 mg/mL and 100 mg/mL treatments, respectively. The treatment effect ($P < 0.0001$) observed was associated with a decrease in ROS production on cells treated with 100 mg/mL (0.56 ± 0.04) compared with the control ($P < 0.0001$, 1.00 ± 0.04), H₂O₂ ($P < 0.0001$, 1.08 ± 0.04), 50 mg/mL ($P = 0.02$, 0.68 ± 0.04), and 50 mg/mL + H₂O₂ ($P < 0.0001$, 0.81 ± 0.04). There was no difference ($P = 0.36$) between 100 mg/mL and 100 mg/mL + H₂O₂ treatments, however, cells treated with 100 mg/mL + H₂O₂ (0.61 ± 0.04) had lower ROS than control and H₂O₂ cells ($P < 0.0001$). Moreover, the 50 mg/mL group had lower ($P < 0.0001$) ROS than the control, H₂O₂, and 50 mg/mL + H₂O₂ ($P = 0.02$), while 50 mg/mL + H₂O₂ also had lower ROS than control and H₂O₂. A trend ($P = 0.10$) was observed for higher ROS in H₂O₂ treated cells than in control. These results suggest that blackberry PC effectively mitigate OS in a dose-dependent manner, even under H₂O₂-induced stress, supporting their potential use in dairy systems.

Key Words: blackberry, oxidative stress, phenolic compounds

1345 The biofilm formation capability of *Staphylococcus* spp. from goat milk. B. Nascimento de Araujo³, A. Della Libera², M. Matiuuzzi³, G. do E³, and L. B. da Costa^{*1}, ¹The Ohio State University, Columbus, OH, ²University of São Paulo, São Paulo, SP, Brazil, ³Federal University of Vale of São Francisco, Petrolina, PE, Brazil.

Staphylococcus spp. are the most prevalent causative agents of caprine mastitis that have developed strategies to ensure their colonization, invasion, and survival in the host, such as biofilm production. Therefore, this study aimed to quantify the biofilm production by *Staphylococcus* spp. isolates from goat milk and evaluate a possible association with the California Mastitis Test (CMT) scores. A convenience sample of 35 *Staphylococcus* spp. isolates, obtained from subclinical cases across 3 Ohio farms, were confirmed using MALDI-TOF-MS. Biofilm

formation (BF) of these isolates was assessed using the microtiter plate adherence test, quantified, and analyzed alongside the CMT scores. The association between these 2 evaluations was identified using Fisher's exact test of independence, with a significance level of 5%. Of the 35 isolates analyzed, 57% exhibited weak biofilm formation, with optical density values ranging from 0.058 to 0.143 nm. A total of 20% of the isolates formed moderate biofilms, whereas 23% of the isolates were classified as strong biofilm formers. The Fisher's exact test revealed a significant association between CMT and BF ($P < 0.01$). A numerical trend was observed in animals with CMT scores of 0 and 1, 15 out of the 22 isolates exhibiting this characteristic were weak biofilm producers. Similarly, isolates that demonstrated moderate biofilm formation (57% of the isolates) were numerically associated with higher CMT scores (4), which indicates greater inflammation linked to advanced or chronic infections. The capacity for biofilm formation, especially in isolates classified as strong, may be associated with greater bacterial persistence in the milking and milk processing environment, increasing the risk of persistent infections. Thus, biofilm formation may contribute to the chronicity of subclinical mastitis, suggesting it is an important pathogenic mechanism that allows bacteria to survive in the mammary environment for prolonged periods. The present study suggests a possible relationship between the biofilm-forming capacity of *Staphylococcus* spp. and the stage of mastitis in goats.

Key Words: biofilm, *Staphylococcus aureus*, goat

1346 Effectiveness of a plant-derived product to improve udder health in organic dairy cows. B. Shrestha^{*1}, R. Neupane¹, B. Whitney^{1,2}, J. Piñeiro¹, and S. Paudyal¹, ¹Department of Animal Science, Texas A&M University, College Station, TX, ²Texas A&M AgriLife Extension, Stephenville, TX.

The objective of this study was to evaluate the effectiveness of a plant-derived bolus for improvement of udder health in organic dairy cattle. A total of 218 multiparous Holstein crossbred lactating cows with SCC >300,000 from a commercial organic dairy farm in central Texas were enrolled in the study. Cows were assigned randomly to the treatment groups; treatment (TRT; n = 104), and control (CON; n = 114), blocked by parity and stage of lactation. Cows in the TRT group were supplemented with 2 boluses of plant-based derived rumen product that the manufacturer claims to disrupt the quorum sensing mechanism of bacterial multiplication (AHV Rapid and AHV Extend; AHV). Cows in the CON group did not receive any intervention. Composite milk samples from enrolled cows were collected on d 0 and d 30 for the analysis of SCC and bacterial population dynamics. Cow-level milk electrical conductivity (EC) and yield (MY) per day were collected from the Afimilk, and rumination time (RT) and water intake (WI) per cow per day were obtained using the SmaXtec rumen-based devices on farm. The effect of treatment on the continuous outcome variables was analyzed using the MIXED procedure in SAS version 9.3 with cow as random effect in the model. The pretreatment EC, MY, RT, and WI were used as a covariate during the analysis. The effect on cow survival was evaluated by Cox's proportional hazard using the PHREG procedure. The TRT group had lower linear scores of SCC than the CON (5.8 ± 0.17 vs. 6.8 ± 0.16; $P < 0.001$) on d 30, particularly in late lactation cows and those infected with contagious pathogens (n = 68). The bacterial growth (log cfu) was lower in TRT (3.3 ± 0.05 vs. 3.4 ± 0.05 cfu/mL; $P < 0.01$) than in CON. The average EC/cow per day was lower in TRT (9.3 ± 0.06 vs. 9.6 ± 0.06; $P < 0.01$) compared with CON. No overall significant effect of treatment was observed on MY and WI between the treatment groups. However, cows infected with contagious mastitis-causing pathogens had higher MY (21.5 ± 0.55 vs. 20.0 ± 0.52 kg; $P < 0.01$) and WI (94.6 ± 1.06 vs.

91.1 ± 0.97 L/d; $P < 0.01$) compared with CON. Survival of cows did not differ among the treatment groups. We conclude that plant-derived products improved udder health while demonstrating no negative effects on the RT and WI, indicating a potential for use in organic dairy farms for improving mammary health.

Key Words: mastitis, organic dairy, plant-derived

1347 Producers' motivations for internal teat sealant use and selective dry cow therapy on automated milking system farms in Ontario—A focus group study. F. G. Guardado Servellon^{*1}, D. L. Renaud¹, M. W. Brunt¹, T. J. DeVries², K. L. Spence¹, and R. Couto Serrenho¹, ¹*Population Medicine, University of Guelph, Guelph, ON, Canada*, ²*Animal Biosciences, University of Guelph, Guelph, ON, Canada*.

In Canada, there is a growing trend among dairy farms to adopt automated milking systems (AMS). However, there is a reluctance to implement some dry-off strategies, such as internal teat sealant (ITS) and selective dry-cow therapy (SCDT), on AMS farms. The objective of this qualitative study was to explore Ontario AMS producers' motivations for the use of ITS and adoption of SDCT as part of their dry-off protocol. Producers were recruited through veterinary clinics and AMS dealerships. Between December 2024 and January 2025, we conducted 4 focus groups with 21 producers (16 using and 5 not using ITS; 4 using and 17 not using SDCT) using different AMS brands. Conversations were recorded and transcribed verbatim using a transcription software (Otter.ai). Data were imported into NVivo and analyzed using applied thematic analysis. Three themes were described from the data, perceived barriers, extrinsic and intrinsic motivations, and information availability. Participants mentioned ITS residues and the consequent risk of AMS mechanical issues as the main barriers for its use. Further, participants not using ITS mentioned a perceived lack of benefit to using ITS. All groups discussed external (e.g., advice from the veterinarian) and internal motivations (e.g., perceived benefit as a preventive measure) for the use of ITS. Regarding SCDT, all groups highlighted the perceived risk of implementation (e.g., increase in SCC and mastitis risk) and suboptimal farm conditions (e.g., bedding material) as reasons for not implementing it. Participants suggested lack of information availability (e.g., no research relevant to their reality) influenced their dry-off practices. Our results suggest that ITS residues are the main barrier faced by AMS producers for the use of ITS as part of their dry-off protocols. The opinion of veterinarians contributes to the producers' decision about ITS use. Regarding SDCT, participants not using SDCT perceive its risks to be greater than its potential benefits.

Key Words: dairy cattle, robotic milking, qualitative research

1348 US dairy producers' perceptions of milk quality and antimicrobial use at dry-off. T. J. Almand^{*1}, D. Bruno², E. Okello¹, and F. S. Lima¹, ¹*Department of Population Health and Reproduction, University of California, Davis, Davis, CA*, ²*Cooperative Extension, University of California Agriculture and Natural Resources, Fresno, CA*.

Mastitis is the most prevalent and costly disease in dairy cattle, with blanket dry cow therapy (BDCT)—routine intramammary antibiotic use at dry-off—being the primary contributor to antimicrobial use in US dairy herds. Selective dry cow therapy (SDCT), which treats only at-risk cows, offers a targeted alternative, yet adoption remains limited. This study surveyed US dairy producers on milk quality and antimicrobial use at dry-off, hypothesizing that perceptions and practices vary by herd size, management, location, and veterinary access.

A nationwide survey, distributed through dairy extension personnel, targeted ~1,000 producers and managers, with an expected response rate of at least 300. The survey assessed (1) the perceived impact of SDCT on herd health, reproduction, milk yield, culling, and economics; (2) mastitis risk awareness; (3) dry-off practices; and (4) concerns about antimicrobial resistance (AMR) and SDCT adoption barriers. Descriptive statistics, including means, standard deviations, medians, interquartile ranges, and percentages, were calculated using the MEANS and FREQ procedures in SAS 9.4. Preliminary results ($n = 123$) indicate that 23% of respondents currently use SDCT. Most were part owners or managers of farms milking <1,000 or >6,000 cows twice daily, with annual yields of 9,000 to 10,000 kg. Among respondents, 67% abruptly cease milking at dry-off, 63% maintain a 45- to 60-d dry period, and 71% use a single treatment protocol for gram-positive, gram-negative, and mixed infections. Additionally, over 50% of farms do not conduct monthly SCC testing. Producers cited milk quality information and SDCT implementation guidance as key influences on adoption. These findings highlight opportunities for targeted education and advisory support to enhance antimicrobial stewardship and promote sustainable mastitis management in US dairy herds.

Key Words: antimicrobial resistance, mastitis, antibiotic usage

1349 Characteristics, culling, and milk yield of cows with clinical mastitis on large dairy farms that have a no-lactational-antibiotic treatment policy: Preliminary results. L. C. B. Juliano^{*}, J. Strickland, L. L. de Souza, I. Buchanna, and P. L. Ruegg, *Michigan State University, East Lansing, MI*.

Some farms have adopted a no-antibiotic treatment policy for clinical mastitis (CM) cases. This analysis aims to describe characteristics, culling, and milk yield of cows with CM on farms that use this approach. Conventional ($n = 3$) and organic ($n = 1$) farms with 12,200 Holstein cows were enrolled from May 2024 to January 2025. Trained milking technicians detected CM and collected a milk sample that was processed at Michigan State University. Statistical analysis was performed on SAS. Chi-squared determined associations and odds of culling for CM cases based on severity. Linear regression assessed relationship between monthly price of cull cows and culling rate. Mixed models assessed the impact of CM on projected 305-d milk yield (M305) before and after case. Survival analysis stratified by farm determined the probability of remaining in the herd after CM. Herds milked an average of 3,230 cows ($\pm 1,266$ SE) and were housed in freestalls with sand bedding. Average yield was 37 kg/cow per day (± 1.8 SE), and bulk tank SCC was 138,000 cells/mL. During the 9-mo study period, 181 CM cases were detected (incidence of <1%/mo), of which 60% were scored as nonsevere and 40% severe. Lower incidence of CM and a greater proportion of severe cases suggest underreporting of mild cases. Mastitis

Table 1 (Abstr. 1349). Cull rate and survival time by farm ($N = 181$; $P < 0.001$)

Farm	Cases ¹	Proportion of culled cases		Survival in the herd after CM (d)		
		N	% ²	Mean	SE	Median
1	122	95	87	14	2.55	1
2	49	14	13	76	5.38	90
3 and 4	10	0	—	—	—	—
Total	181	109	100	90 ³	—	—

¹Total CM cases detected between May 2024 and January 2025.

²Percentage of column total.

³Total follow-up days.

cases were distributed as gram-negative (60%), no bacterial growth (19%), and gram-positive (21%). Many gram-negative cases suggest that few cases would benefit from antimicrobial therapy. Overall, 60% of cows were culled (Table 1), but farm 1 was 12 times more likely to cull cases compared with other farms ($P < 0.001$). Monthly price of cull cows (\$4.13/kg $\pm$ 0.04) was associated with monthly culling rate ($R^2 = 0.31$; $P = 0.07$). After CM, the M305 was reduced by 547 kg ($P = 0.04$), resulting in a loss of about \$270 of milk income/cow per lactation. High beef prices have been shown to drive culling decisions, and based on etiology, few cases would benefit from antibiotic treatment. Future studies will compare the economic impact of this approach with that of treated cows.

Key Words: culling, mastitis, milk yield

1350 Associations between mastitis incidence and risk factors in US organic-certified dairy farms. C. Nino De Guzman^{*1}, L. Prada², S. Bellinzoni², P. Muñoz³, C. Hernandez³, C. Ibarguren³, R. Weng³, Q. Kolar⁴, V. E. Cabrera⁵, B. J. Heins⁶, R. Lynch⁴, E. K. Miller-Cushon¹, G. M. Schuenemann², P. Pinedo³, and A. De Vries¹, ¹University of Florida, Gainesville, FL, ²The Ohio State University, Columbus, OH, ³Colorado State University, Fort Collins, CO, ⁴Cornell University, Ithaca, NY, ⁵University of Wisconsin, Madison, WI, ⁶University of Minnesota, Morris, MN.

The objective of this study was to assess the association between mastitis incidence and self-reported risk factors in organic dairy farms in the United States. Through farm visits and questionnaires, we surveyed 48 organic farms: 25 in Ohio, 10 in New York, 5 in California, 4 in Colorado, 3 in Texas, and 1 in Georgia. The average reported annual mastitis incidence was 13% $\pm$ 9. The median milking herd size was 74 cows (range: 22–4081). The average daily milk yield was 22 $\pm$ 6 kg/cow. Farms were categorized by region as West (19%), Central (6%), and East (75%). The proportions of cows by region relative to the number of cows in total were 56% for the West, 31% for the Central, and 13% for the East. Farms were categorized as small (<100 cows, 62%), medium (100–1,000 cows, 21%), and large (>1,000 cows, 17%). Explanatory variables were analyzed using the “lm” function of R Studio. Region and farm size were included in all statistical models as control variables. Tested individually, variables with a $P \leq 0.20$ were added to the final model. The final model included the variables milk yield, the importance of vaccines to control mastitis, the concern threshold for bulk tank SCC, the concern threshold for mastitis cases in a single day, the number of towels used to clean udder before attachment, cluster removal method, and use of vaccines. An increase in annual mastitis incidence was associated with milk yield (+0.5% $\pm$ 0.2)/kg and perception of the importance of vaccination (very important: +18% $\pm$ 9), possibly due to a greater use of vaccines in farms where mastitis is a problem. A decrease in annual mastitis incidence was associated with the concern threshold for bulk tank SCC (–5% $\pm$ 2)/100,000 cells/mL. In conclusion, only 3 explanatory variables were associated with annual mastitis incidence. Further research will include associations of mastitis with milking procedures, cow hygiene, management, and facilities.

Key Words: mastitis, milk quality, organic

1351 Microbiome dynamics associated with mastitis-causing bacteria in different hotspots within organic and conventional dairy farms. B. Shrestha^{*}, R. Neupane, S. Chitlapilly Dass, K. Mani, V. Palanisamy, Z. Vice, and S. Paudyal, Texas A&M University, Department of Animal Science, College Station, TX.

The objective was to characterize diversity and relative abundance (RA) of microbiomes associated with mastitis-causing bacteria in different pathogen hotspots within organic (ORG) and conventional dairy farms (CON). Representative CON and ORG dairy farms in Texas were selected and cross-sectional samples from bedding (SAN, $n = 24$), water (WAT, 26), feed area (FED, 18), milking parlor floor (MAT, 12), teats before predip (TBP, 22), teats after predip (TAP, 22), milking unit before milking (BML, 4) and after milking (AML, 22), cow milk samples (MLK, 22), and bulk milk tank (BMT, 4) were collected. The genomic DNA extraction for all swab samples were performed using DNeasy PowerSoil Pro Kit and milk samples were performed using DNeasy Blood & Tissue Kit (Qiagen, Germany). The extracted DNA was quantified, followed by library preparation and sequencing on the DNBSEQ platform. The obtained raw shotgun metagenomic sequences were quality-filtered and taxonomically annotated for mastitis-causing bacteria using the Kraken2v2.1.2 tool. Nonparametric ANOVA in SAS 9.4 was used for statistical analysis of bacterial abundance. The phyla *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Tenericutes* were observed with RA of 61%, 34%, 4%, and 0.02% in ORG compared with 88%, 5.8%, 6%, and 0.03%, respectively, in CON. Overall, RA of *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Streptococcus uberis* were not statistically different ($P > 0.05$) between farms, indicating minimal variation in the major contagious mastitis pathogens. However, RA for *Streptococcus dysgalactiae* (0.015% $\pm$ 0.002% vs. 0.012% $\pm$ 0.004%, $P < 0.05$), *Corynebacterium sp* (14.8% $\pm$ 2.18% vs. 10.8% $\pm$ 2.03%, $P < 0.05$), *Staphylococcus chromogenes* (0.13% $\pm$ 0.03% vs. 0.05% $\pm$ 0.02%, $P < 0.0001$), and *Escheria coli* (1.1% $\pm$ 0.41% vs. 0.45% $\pm$ 0.18%, $P < 0.0001$) were higher in CON compared with ORG. The RA of *Proteobacteria* in ORG was highest in WAT (97.3% $\pm$ 0.51%) than FED (72.7% $\pm$ 7.74%, $P < 0.01$), MAT (75.4% $\pm$ 10.77%, $P < 0.05$), BML (67.9% $\pm$ 8.46%, $P < 0.05$), SAN (29.4% $\pm$ 3.46%, $P < 0.001$), TBP (33.8% $\pm$ 7.70%, $P < 0.001$), and MLK (4.9% $\pm$ 0.55%, $P < 0.001$). In CON, the RA of *Proteobacteria* was higher in LIN (98.4% $\pm$ 0.67%) than SAN (75.1% $\pm$ 2.32%, $P < 0.05$), TAP (73.9% $\pm$ 5.53%, $P < 0.05$), WAT (65.7% $\pm$ 2.48%, $P < 0.05$), MAT (32.7% $\pm$ 4.7%, $P < 0.05$), FED (10.9% $\pm$ 1.99%, $P < 0.05$), and MLK (6.02% $\pm$ 0.96%, $P < 0.05$). These results suggest a difference in mastitis-causing pathogens microbiome in different hotspots in the organic and conventional dairy farming systems with opportunities for targeted intervention.

Key Words: microbiome, mastitis, dairy farm

1352 Dynamics of culling risk in organic dairy herds in the United States. B. Shrestha^{*}, R. Neupane¹, P. Pinedo², J. Piñeiro¹, and S. Paudyal¹, ¹Department of Animal Science, Texas A&M University, College Station, TX, ²Department of Animal Sciences, Colorado State University, Fort Collins, CO.

The objective was to understand the culling dynamics with reported disposal reason for the cows in organic dairy herds. A total of 160,667 lactation records were obtained from DHIA records for cows calving between 2018 and 2022 in 483 certified organic dairy herds from 38 states in the United States. Nine disposal codes including feet and legs, low production, injury or other, mastitis, disease, udder problems, died, and reason not reported were considered as codes of interest. The distribution of culled cows by disposal code was estimated by parity (1, 2, 3, and ≥ 4), cow-relative 305-d mature equivalent milk yield (low, intermediate, and high), herd-relative 305-d mature equivalent milk yield (low: < mean – SD; medium mean – SD to mean + SD; high: > mean + SD), herd size (small: 0–99, medium: 100–999, and large: >999). Annualized live culling rate and death rate were 25% and 4.7%, respectively. The primary disposal codes were reproduction problem

(RPRO; 27% of all culling), followed by died (DEAD; 16%), mastitis (MAST; 13%) and low production (PROD; 13%), and injury or other (12%). The annualized live and death culling rates differed by year. The odds were analyzed using binary logistic regression (Proc Logistic in SAS 9.4). The odds of culling due to DEAD were lower in large than in small farms (odds ratio [95% CI] = 0.62 [0.59–0.65]), culling due to RPRO was lower in medium than in small farms (0.94 [0.90–0.99]), culling due to MAST was lower for large (0.56 [0.53–0.59]) and medium (0.56 [0.53–0.59]) farms than for small farms. The odds of culling due to MAST were lower in farms with high (0.34 [0.30–0.38]) and intermediate (0.65 [0.62–0.69]) cow relative 305 ME milk yield as compared with farms with low milk yield. The odds of culling with the

RPRO and MAST were lower in farm having high (0.38 [0.34–0.42] and 0.40 [0.35–0.46]) and intermediate (0.41 [0.39–0.43] and 0.81 [0.77–0.85]) herd relative 305 ME milk yield than in farms with low herd relative milk yield. The odds of DEAD and culling due to MAST were lower in parity 1 (0.86 [0.82–0.9] and 0.66 [0.63–0.70]), parity 2 (0.85 [0.81–0.90] and 0.71 [0.67–0.75]), and parity 3 (0.92 [0.87–0.97] and 0.88 [0.83–0.94]) as compared with the parity ≥ 4 . In conclusion, the risk of culling by disposal code varied with parity, cow and herd relative milk yield, and herd size.

Key Words: organic dairy, culling, disposal code

Physiology and Endocrinology 1

1353 Impact of prepartum skeletal muscle reserves on early lactation performance in primiparous dairy cows. R. K. Coombe*, Q. L. Rosenthal, H. U. Rehman, J. F. Markworth, and J. P. Boerman, *Department of Animal Sciences, Purdue University, West Lafayette, IN.*

The objective of this study was to characterize the extent of skeletal muscle mobilization in primiparous dairy cattle as they transition into their first lactation. Twenty-one heifers were divided into 2 groups, high muscle (HM, >5.28 cm, $n = 10$) and low muscle (LM, <5.28 cm, $n = 11$) based on an ultrasound measurement of the depth of the *longissimus dorsi* (LD) muscle on the day of enrollment in the study between 35 and 42 d before expected calving. Weekly measurements of LD muscle depth, BW, and BCS were recorded from enrollment until 28 DIM. Milk weights were recorded daily, and milk components were measured weekly. Data were analyzed in SAS v9.4 and the statistical model for repeated measurements included the fixed effects of group, week, their interaction, and the random effect of cow within group. The statistical model for single measurements per animal included the fixed effect of group and when appropriate included age at first calving and sex of calf as a covariate. There was no effect of group for milk, milk fat, milk protein, or ECM yields (all, $P > 0.20$). During the prepartum period HM cows mobilized 0.26 cm of LD muscle, whereas the LM cows accreted 0.41 cm of LD muscle ($P = 0.02$). In the postpartum period, there was no difference in the muscle mobilization between the 2 groups, with both groups losing 0.42 cm of muscle from 1 to 4 wk in lactation ($P = 0.67$). By 4 wk in lactation, HM cows averaged 0.49 cm more muscle than LM cows (5.14 vs. 4.64 cm; $P = 0.05$). The HM cows lost 26 kg from 1 to 4 wk in lactation, whereas LM cows lost 46 kg in the same time ($P = 0.05$). There was no difference in calf birthweight, colostrum yield, or colostrum Brix value between groups ($P < 0.30$). Overall, primiparous animals with greater muscle reserves mobilized muscle prepartum but at 4 wk into lactation still had greater muscle reserves. Muscle reserves did not affect any calf variables measured or milk production in early lactation for primiparous cows.

Key Words: *longissimus dorsi*, muscle reserves, primiparous

1354 Parity and body weight loss effects on tissue mobilization, milk production, and blood metabolites during early lactation. M. L. Simonds* and J. P. Boerman, *Department of Animal Sciences, Purdue University, West Lafayette, IN.*

Determining how and to what extent dairy cattle, across parity, allocate body reserves to support the physiological demands of lactation will enable producers to implement precision management strategies that optimize milk production efficiency and health. Our first objective was to compare muscle and backfat tissue reserves between early lactation primiparous and multiparous dairy cows. Our second objective was to examine the relationship between BW loss and muscle and adipose tissue mobilization in early lactation. Cows were categorized by parity (primiparous, $n = 14$; multiparous, $n = 26$). Independent of parity, cows were assigned to BW loss group based on percent of total BW loss from 3 to 31 DIM, either low BW loss (LBWL; <6.0%; $n = 17$) or high BW loss (HBWL; >6.0%; $n = 23$). Milk and blood samples, BW, BCS, and ultrasound measurements were taken on 3, 10, 17, 24, and 31 DIM. Milk yield was recorded, and samples were taken from both milkings on each sampling day. Blood samples were taken to measure glucose, BHB, plasma insulin, 3-methylhistidine (3-MH) and creati-

nine concentrations. At each time point, 3 ultrasound images above the *longissimus dorsi* were collected to quantify muscle and backfat depth and change in depth from 3 to 31 DIM. The statistical model included the fixed effects of parity, BW loss group, DIM, and parity or BW loss group by DIM interactions, and the random effect of cow nested within parity and BW loss group. Multiparous cows had greater milk, milk protein, and milk fat yields compared with primiparous cows (all $P < 0.05$); however, BW loss did not affect milk production variables (all $P > 0.10$). Primiparous cows had higher blood glucose (+6.4 mg/dL; $P < 0.05$) and greater loss in backfat depth ($P = 0.07$) compared with multiparous cows. There were no BW loss effects on muscle or backfat depth change, blood glucose, 3-MH, and creatinine concentrations (all $P > 0.10$). There was no effect of parity on muscle depth in early lactation ($P = 0.48$), regardless of parity muscle depth decreased from 4.77 cm on d 3 to 4.07 d 31 ($P < 0.01$). Additionally, because there was no effect of BW loss on the amount of muscle or backfat depth change in early lactation, BW loss was not deemed to be a strong predictor of muscle or backfat mobilization in early lactation.

Key Words: primiparous, body weight loss, tissue reserves

1355 Characterizing and analyzing differences in the bovine uterine microbiome as a reflection of breed and return to estrus postpartum. S. Dupont*, S. Johnson², and J. Bohlen¹, ¹University of Georgia, Athens, GA, ²DeLaval, Gurnee, IL.

Over 20% of dairy cattle experience uterine microbial disease in the postpartum period, contributing significantly to reduced reproductive efficiency. This study aimed to explore the uterine microbiome in both Holstein and Jersey cattle and any implications it has for resumption of estrus postpartum. Holstein ($n = 21$) and Jersey ($n = 20$) cows of various lactations were swabbed by 2 sterilized, double-guarded, culture swabs (Jorgenson Labs, Loveland, CO) in the uterine body between 45 and 60 DIM for uterine microbiome analyses. Samples were extracted using Qiagen QIAamp BiOstic Bacteremia DNA Kit and stored in -80°C for future analyzation. Samples were shipped to Element Biosciences for Element LoopSeq Long-Read 16S ribosomal ribonucleic acid (rRNA) Gene Sequencing. The duplicate sampling method allowed uterine microbiomes to be compared as (1) Holstein (HO) versus Jersey (JE), and (2) normal (N), <45 DIM, versus delayed (D), >45 DIM, resumption of estrus postpartum. Sequencing results were viewed broadly as phylum through subsequent taxa to species. The most abundant phyla present in the uterine microbiome in all animals were *Fusobacteriota*, *Bacteroidota*, *Actinobacteriota*, and *Firmicutes*. *Fusobacteriota* ($P = 0.02$) as well as *Bacteroidota* ($P = 0.04$) were found in greater abundance in HO, whereas a greater number of JE samples were comprised of *Actinobacteriota* ($P = 0.04$). When taxa level moved to family, the major difference was that JE samples contained a higher percentage of *Propionibacteriaceae* ($P = 0.01$). There was no difference in the Alpha Diversity box plot ($P = 0.057$) for HO versus JE, nor in N versus D cows ($P = 0.38$). *Actinobacteria* occurred in greater quantities in N cows ($P = 0.02$), which are frequently identified in healthy uteri. *Clostridia* had greater abundance in D cows ($P = 0.02$). When analyzed, most microbial species present in the uterine microbiome were commensal, however some had the potential to become pathogenic. Such pathogenic species, *Actinomyces pyogenes* ($P = 0.04$) and *Fusobacterium necrophorum* ($P = 0.04$), were only found in D cows. These results add to the body of work involving the uterine microbiome by characterizing breed differ-

ences as well as identifying differences in microbial abundance relative to resumption of estrus postpartum.

Key Words: uterine microbiome, estrus expression, breed

1356 Dexamethasone affects energy metabolism and inflammation following immune activation in Holstein cows. G. J. Combs^{*1}, E. J. Mayorga¹, B. M. Buol¹, S. Rodríguez-Jiménez¹, L. C. Bombeck¹, T. A. Flemming¹, S. O'Connell², and L. H. Baumgard¹, ¹Department of Animal Science, Iowa State University, Ames, IA, ²Celtic Sea Minerals, Marigot Research Centre, Clash, Tralee, Co. Kerry, Ireland.

Objectives were to evaluate the effects of dexamethasone (DEX) on inflammation and metabolism following an i.v. LPS challenge. Holstein cows ($n = 23$; parity 2.6 ± 0.7 ; 143 ± 4 DIM) were randomly assigned to receive an i.m. injection of either (1) saline (SAL; ~ 13 mL; $n = 11$) or (2) DEX (0.04 mg/kg BW; $n = 12$) 8 h before an i.v. LPS bolus (0.09 μ g/kg BW). Data were analyzed with PROC MIXED of SAS. Before LPS bolus, DEX increased vaginal temperature (0.4°C), blood glucose (37%), insulin (3.0-fold), and white blood cell (WBC) counts (1.8-fold) relative to SAL. The LPS reduced DMI on d 1, and DEX attenuated the decline (20.9 vs. 38.1%; $P < 0.01$). The LPS decreased milk production (40%) on d 1 similarly between treatments. Pyrexia occurred post-LPS, but DEX blunted this response (0.7 vs. 1.3°C ; $P < 0.01$). Blood Ca^{2+} decreased 33% at 6 h following LPS in SAL but only 5% in DEX. The LPS caused transient hyperglycemia (43% at 0.5 h) and hyperinsulinemia (2.9-fold at 3 h) in SAL, but DEX-administered cows were hyperglycemic and hyperinsulinemic through 48 h ($P < 0.01$). The LPS caused hyperglucagonemia (49% at 3 h) only in SAL. The LPS increased BUN, which DEX abrogated. The LPS decreased circulating NEFA and BHB (32% and 50%, respectively) in SAL, while DEX abrogated BHB changes and enhanced the NEFA decline ($P < 0.01$). The LPS caused hypercortisolemia through 3 h, which was eliminated by DEX (182 vs. 31 ng/mL per hour; $P < 0.01$). WBC counts declined following LPS (nadir ≤ 3 h following LPS), which DEX attenuated (71% vs. 81%) and DEX increased WBC counts at nadir ($P < 0.01$). Furthermore, DEX blunted LPS-induced neutropenia and accelerated leukocytosis from 6 h on ($P < 0.01$). The LPS-binding protein and serum amyloid A increased following LPS, but peak values were decreased in DEX (52 and 76%, respectively), while haptoglobin increased similarly. Interferon-gamma (IFN- γ), tumor necrosis factor- α , and IL-6 increased following LPS ($P < 0.01$), as did chemokines IFN- γ -induced protein 10, macrophage inflammatory protein-1 α , and monocyte chemoattractant protein-1, but these were blunted in DEX. In summary, DEX inhibited inflammation and caused insulin resistance but did not fully rescue decreased productivity.

Key Words: LPS, glucocorticoids

1357 Low rumen pH and high cellulolytic bacteria abundance promote virulent LPS synthesis and systemic inflammation in dairy cows. G. Hou^{*1}, D. Gao¹, Y. Zhuang¹, Y. Xu¹, W. Jiang^{1,2}, T. Chen¹, S. Liu¹, S. Li¹, M. Ma¹, and Z. Cao¹, ¹State Key Laboratory of Animal Nutrition and Feeding, International Calf and Heifer Organization, College of Animal Science and Technology, China Agricultural University, Beijing, China, ²College of Animal Science, Xinjiang Agricultural University, Urumqi, Xinjiang Uyghur Autonomous Region, China.

This study aimed to investigate the differences in blood immune parameters and rumen microbiota in Holstein cows with varying rumen fluid pH and cellulolytic bacteria (CB, including *Ruminococcus flavefaciens*,

Ruminococcus albus, *Fibrobacter succinogenes*, and *Butyrivibrio fibrisolvens*) levels during early lactation. These cows were fed the same diet from 1 to 21 DIM and milk yield was recorded daily. At 21 DIM, milk samples were collected before morning feeding and rumen fluid and blood samples were collected 2 to 4 h after morning feeding. Sixty cows were divided into 4 groups based on rumen pH and CB levels (quantified via quantitative real-time PCR): low pH, low CB (LPLC, $n = 15$; pH ≤ 6.0 , $\log_{10}\text{CB} < 9.20$), low pH, high CB (LPHC, $n = 15$; pH ≤ 6.0 , $\log_{10}\text{CB} > 9.20$), high pH, low CB (HPLC, $n = 15$; pH ≥ 6.5 , $\log_{10}\text{CB} < 9.20$) and high pH, high CB (HPHC, $n = 15$; pH ≥ 6.5 , $\log_{10}\text{CB} > 9.20$). Data were analyzed by 2-way ANOVA of IBM SPSS Statistics 26.0. Spearman correlation analysis was conducted. The results showed that low pH was associated with elevated acetate, butyrate, and total VFA concentrations ($P < 0.05$), as well as increased pro-inflammatory cytokines (IL-1 β , IL-8, TNF- α , IFN- γ , and MCP-1), LPS, histamine, and malondialdehyde, alongside reduced antioxidant capacity and immunoglobulins (IgA, IgM, IgG; $P < 0.05$). High CB abundance correlated with increased relative abundance of *Succinivibriomaceae_UCG-001* and hexa-acylated LPS synthesis enzyme *LpxM* ($P < 0.05$), and *LpxM* showed a strong positive correlation with *Succinivibriomaceae_UCG-001* ($r = 0.99$, $P < 0.01$). Notably, the LPHC group exhibited higher *Succinivibriomaceae_UCG-001* and *LpxM* compared with LPLC ($P < 0.05$), whereas no difference was observed in penta-acylated LPS synthase *LpxL* ($P = 0.086$). These findings suggest that under low rumen pH conditions, higher CB abundance may promote the synthesis of hexa-acylated LPS (a more virulent form) potentially increasing the risk of SARA and systemic inflammation in dairy cows.

Key Words: ruminal pH, cellulolytic bacteria, inflammation

1358 Endotoxemia alters plasma endocannabinoid and ethanolamide profiles in dairy cows. M. N. Myers^{*1}, M. Chirivi¹, J. Parales Girón², J. M. dos Santos Neto², L. C. Worden², A. L. Lock², and G. A. Contreras¹, ¹Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI, ²Department of Animal Science, College of Agriculture and Natural Resources, Michigan State University, East Lansing, MI.

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

Endotoxemia leads to systemic inflammation, metabolic disturbances, and production losses. Endocannabinoids (eCB) are lipid-based molecules that regulate key metabolic and inflammatory pathways. Endotoxemia and energy deficits drive synthesis of other lipid-based mediators, yet their effects on eCB are unknown. The objective of our 28-d study was to investigate how single and repeat endotoxin exposures during negative energy balance (NEB) modulate circulating eCB levels in vivo. Multiparous, lactating cows ($n = 32$; avg. 225 DIM) were assigned to 1 of 4 treatments (SAL-SAL, SAL-LPS, LPS-SAL, LPS-LPS; $n = 8/\text{group}$) in a 2×2 factorial design. Two 4-d feed restriction periods (initiated on d 8 and d 20) targeted a -15 Mcal energy balance and treatments (SAL or LPS) were infused via jugular catheter over 20 min on d 10 and 22 (SAL: 100 mL sterile saline; LPS: 100 mL SAL+1 μ g LPS/kg BW). Plasma eCB were quantified via LC-MS/MS at enrollment, immediately before, and 4 h after infusions. Linear mixed models were constructed for data analysis. All differences, reported as percent difference in LPS versus SAL, are $P < 0.05$. Feed restriction increased plasma levels of 2-arachidonoylglycerol (2-AG) by 55% and anandamide (AEA) by 15%. Four hours after the first LPS infusion, oleoylethanolamide (OEA) increased by 22% and 2-AG levels dropped by 6%. Anandamide and linoleoylethanolamide (LEA) increased by 26% and 7%, respectively, for 12 d after LPS exposure. Following infusion 2, 2-AG increased by

26% in SAL-LPS; stearoylethanolamide increased by 25% in LPS-LPS; palmitoylethanolamide increased by 14% in LPS-SAL; and in SAL-LPS and LPS-LPS, respectively, AEA (13%, 8%), LEA (30%, 15%), and OEA (44%, 52%) declined. Our findings indicate endotoxemia and NEB dynamically alter eCB profiles, with repeated challenges amplifying responses. The sustained elevations in AEA and LEA may imply that endotoxemia and NEB induce adaptive changes in eCB signaling to modulate metabolism and inflammation. Altered eCB levels in response to repeated endotoxin exposures may reflect maladaptive responses that exacerbate inflammation or disrupt energy metabolism, warranting further investigation.

Key Words: endocannabinoids, endotoxemia, inflammation

1359 Effects of cannabinoid receptor 2 ligand beta caryophyllene and heat load on inflammatory response of blood monocytes from dairy cows. Y. Butenko¹, B. Shattenstein^{1,2}, P. dos Santos Silva^{1,2}, L. Hubner^{1,2}, and M. Zachut^{*1}, ¹Volcani Institute, Rishon LeZion, Israel, ²Faculty of Agriculture, Hebrew University, Rehovot, Israel.

Activating the cannabinoid receptor 2 (CNR2) by agonist β -caryophyllene (BCP) exerts anti-inflammatory effects in rodents. We examined the effects of BCP on the inflammatory response of bovine monocytes under thermoneutral (TN) or heat stress (HS) conditions *ex vivo*. Blood monocytes from dairy cows ($n = 7$) were controls (CTL) or supplemented with BCP at 1 mM, and were incubated in TN (38.5°C) or HS (41.5°C). After 24 h, half of the wells were challenged with LPS (1 μ g/mL), and after 2 h RNA was extracted for qPCR. Cells from each cow were divided into all treatments (4 treatments: CTL, BCP, CTL+LPS, BCP+LPS, in TN or HS, overall 8 conditions; with 3 technical replicates). Data were analyzed with PROC MIXED of SAS for effects of BCP, LPS, HS, and interactions. The BCP supplementation did not affect cell viability. The expression of CNR2 was lower in BCP than in controls, and was lower with LPS than in cells without LPS ($P = 0.0003$); the effects of BCP, LPS and their interaction were significant ($P = 0.0004$) both in TN and in HS. The expression of tumor necrosis factor α (TNF α) was greater in BCP+LPS than in CTL, both in TN and in HS ($P = 0.03$). The expression of nuclear factor kappa B subunit 1 (NFKB1) was affected by BCP, LPS and their interaction ($P < 0.0001$); NFKB1 was increased with LPS ($P = 0.002$), but not in BCP compared with controls. The expression of IL-6 (IL6) tended to be affected by BCP ($P = 0.07$), and the expression of IL-10 (IL10) was affected by BCP, LPS, and their interaction ($P = 0.003$); their expressions were increased in controls with LPS but were lower in BCP+LPS cells. The expression of anti-oxidative NAD(P)H dehydrogenase [quinone] 1 (NQO1) was affected by HS, BCP and LPS ($P = 0.04$); in HS, its expression tended to be higher in BCP than in controls. Overall, both LPS and BCP supplemented *ex vivo* to bovine monocytes reduced the relative expression of CNR2 and differentially affected inflammatory and anti-oxidative responses, whereas HS had a minor effect. This indicates that CNR2 is involved in the inflammatory response of bovine monocytes, and that BCP may affect their function via CNR2 regulation.

Key Words: cannabinoid receptor 2, heat stress, immune

1360 Hemogasanalysis patterns modifications in Holstein Friesian and Brown Swiss lactating cows coping with a short-term heat wave. P. De Palo^{*1}, V. Landi¹, E. Trevisi², U. Bernabucci³, C. Lecchi⁴, F. Cecilian⁴, and A. Maggolino¹, ¹University of Bari A. Moro, Italy, Valenzano, Italy, ²Catholic University of the Sacred Heart, Piacenza,

Italy, ³Tuscia University, Viterbo, Italy, ⁴University of Milan, Lodi, Italy.

The study aimed to assess the different hemogasanalysis responses of Brown Swiss (BS) and Holstein Friesian (HF) lactating cows to a natural 4-d summer heatwave. Forty multiparous BS ($n = 20$) and HF ($n = 20$) cows, balanced for DIM, ECM yield, and BCS, were housed together. The barn was provided with 5 data loggers (Hobo Pro Temp probes) placed on the upper support rails of the cubicles. The temperature and relative humidity were used for calculating the THI. Sprinklers and fans were turned off for 4 d during a natural heatwave. Before switching off, the THI values recorded into the barn never exceeded THI values of 72. Respiration rate (RR) was recorded at 0400, 1500, and 2000 h each day. At the same time points, blood samples were collected by the jugular vein for the determination of lactate, blood pH, partial pressure of oxygen (pO₂), partial pressure of carbon dioxide (pCO₂), hydrogen ion activity in blood (pH), base excess in extracellular fluid (BE), bicarbonate level (HCO₃⁻), average oxygen saturation and total carbon dioxide (TCO₂), using an Abbott i-STAT portable analyzer. Vaginal temperature (VT) was recorded continuously (iButton, model DS1922L). All parameters were analyzed by general linear mixed models using PROC MIXED with fixed effects of breed, time, and their interaction. Vaginal temperature confirmed that cows before the switching off were not experiencing heat stress (VT always lower than 39°C); they started coping heat stress (HS) after switch off of the cooling system during the natural heat wave. The pCO₂, TCO₂, HCO₃⁻, and BE were affected by breed, time, and their interaction ($P < 0.001$). The pCO₂ increased during the heat wave, with higher values in HF than BS ($P < 0.01$; pCO₂, 43.27 vs. 35.97 mmHG). TCO₂ and HCO₃⁻ decreased in both the breeds, but with lower values in HF than BS ($P < 0.05$). Furthermore, both these parameters decreased faster in HF than in BS during the heatwave ($P < 0.01$). The BE was positive in both the breeds at the first day and reached negative values up to the fourth HS day, with lower values in HF than in BS ($P < 0.001$: -4.65 vs. -3.15 mEq/L respectively). Both the breeds experienced during the trial the tendency for metabolic acidosis, although with different severity rate.

Key Words: heat stress, hemogasanalysis, heat wave

1361 Cis-9,trans-11 conjugated linoleic acid supports growth but fails to reduce mead acid during essential fatty acid deficiency in CD-1 mice. A. B. Haile^{*}, S. Burtnett, Y. A. Adeniji, and K. J. Harvatin, The Pennsylvania State University.

Linoleic and linolenic acids are essential fatty acids (EFA) crucial for cell membrane integrity and signaling molecules. *Cis-9,trans-11* CLA, produced by microbes and present in ruminant products, has demonstrated bioactive effects on lipid metabolism and inflammation; however, its potential overlapping function as an EFA is not known. We hypothesized that CLA could substitute for EFA and compensate for linoleic acid deficiency. This study evaluated CLA's potential to rescue EFA deficiency compared with linoleic acid (LA) in a mouse model. Essential fatty acid deficiency was induced in CD-1 mice by feeding their dams a deficient diet containing 4% coconut oil from d 2 of lactation. After weaning, pups were placed on a fat-free diet for 1 wk. Rescue treatments began at ~28 d of age, with 20 males and 20 females receiving daily oral doses of 25.7 mg of ethyl esters (23.4 mg FA) of CLA (*cis-9,trans-11*), LA (positive control), oleic acid (OA; energy control), or water (H₂O; negative control) for 14 d. Data were analyzed using a randomized block design to test the effect of treatment. The LA treatment increased growth rate and reduced mead acid in the liver and carcass, confirming its role as an EFA. The CLA treatment increased

growth compared with OA and H₂O but was lower than LA. However, CLA did not reduce mead acid or the triene/tetraene ratio, which are classical markers of EFA deficiency. Conjugated linoleic acid increased a 20:3 isomer, suggesting elongation and desaturation. Although CLA failed to rescue mead acid, it increased growth, suggesting a partial overlap with EFA functions. Further studies are needed to clarify these non-mead acid-related EFA functions of CLA.

Key Words: polyunsaturated fatty acids, arachidonic acid, triene/tetraene ratio

1362 Deciphering the mechanisms underlying the production of dairy fat using mammary epithelial cells. N. Tzirkel-Hancock* and N. Argov-Argaman, *The Hebrew University of Jerusalem, Rehovot, Israel.*

Bovine milk fat consists of a unique fatty acid (FA) composition, rich in short- and medium-chain FA synthesized *de novo* in mammary epithelial cells (MEC) and incorporated into triglycerides. The high concentration of these FA is unique to the ruminant mammary gland and not found in other ruminant tissues. We hypothesized that a distinct expression pattern of lipogenic genes and the distinguished response to lipogenic metabolites (i.e., acetate) drive this distinct profile. To investigate, we determined the response of bovine MEC to acetate in terms of gene expression and FA composition. As a control, we used bovine fibroblasts

as non-milk-producing cells with a lipogenic capacity in culture, which utilize the same lipogenic pathways and express similar genes to those used by MEC. MEC and fibroblasts were supplemented with 40 mM of acetate for 24 h and harvested for lipid or RNA extraction. Genes related to short-chain FA synthesis and activation were targeted with siRNA to reduce their expression. Gene expression was quantified using real-time PCR ($\Delta\Delta C_t$ method). Fatty acid content was measured using GC. Each experiment was conducted at least twice, with 3 replicates per treatment. Results were analyzed using Student's *t*-test or one-way ANOVA, followed by Tukey-Kramer HSD, with significance set at $P < 0.05$. The MEC and fibroblasts exhibited significantly different basal FA composition and gene expression profiles. Under basal conditions, medium-chain capric acid (C10:0) and lauric acid (C12:0) were undetectable in both cell lines. However, acetate induced their synthesis exclusively in MEC, and myristic acid (C14:0) abundance increased 2.2-fold in MEC ($P = 0.007$) but remained unchanged in fibroblasts. Moreover, the expression of acetyl-CoA short-chain synthetase (ACSS1), an enzyme responsible for activation of short-chain FA, was increased 2-fold by acetate in MEC ($P = 0.004$) but not fibroblasts. Reduced expression of ACSS1 in MEC was abolished by acetate supplementation ($P = 0.015$). These findings suggest that intrinsic gene expression patterns and metabolic responses to lipogenic metabolites contribute to the distinctive FA composition of bovine milk.

Key Words: milk fat synthesis, lipogenic genes, short-chain fatty acids

Production, Management, and the Environment Symposium: The Ruminant Farm Systems (RuFaS) Model—A Modular Approach to Research and Decision Support for Sustainable Dairy Farming

1363 The RuFaS animal module: A comprehensive, flexible herd model. K. F. Reed^{*1}, V. E. Cabrera², J. C. Waddell⁴, S. P. HekmatiAthar², A. Liu³, K. R. Briggs⁴, J. S. Adamchick³, H. Hu³, and Y. Gong², ¹USDA-ARS U.S. Dairy Forage Research Center, Madison, WI, ²University of Wisconsin–Madison, Madison, WI, ³Cornell University, Ithaca, NY, ⁴Dairy Management Inc., Rosemont, IL.

The animal module of the Ruminant Farm Systems (RuFaS) model simulates individual animals on a daily basis and offers a wide variety of animal, herd, and dietary options so users can flexibly represent management practices of interest. Each day, the animal module updates the basic physiological status of every animal on the farm including BW, pregnancy status and conceptus weight, and lactation status and milk production according to mechanistic models where available and empirical methods when needed. Milk production is predicted using Woods lactation curve models that are stochastically assigned to each animal based on published regional parameters that are then fit to annual herd milk production. The physiological updates inform management practice algorithms that direct movement of animals between pens, initiate breeding, culling decisions, and feeding. Diets are updated on a user-provided interval and can either be automatically formulated to meet requirements with a least-cost diet or defined by a user-provided recipe. If the provided diet does not meet the expected average nutrient requirements, milk production is reduced up to a user provided threshold. A single diet is fed to each pen before predicting individual animal enteric methane and manure excretion each day. Available farm grown and purchased feeds are provided by the feed storage module and aggregated manure is passed to the manure module, where it enters manure management chains that are assigned to each pen with many to many relationships. Other user options for herd and animal management include specifying reproduction programs for heifers and cows, setting culling rates by parity, breeding and animal sale timing, breed, and BW. Detailed outputs of milk production, animal sales, herd demographics, manure amount and composition, and enteric methane are produced on a daily basis and can be collected and summarized according to the application. The granularity and flexibility of the animal module offer a flexible platform for understanding the impact of animal management on farm-level production efficiency and nutrient cycling.

Key Words: dairy herd, modeling, animal module of the Ruminant Farm Systems (RuFaS)

1364 The RuFaS manure module: Relevance, methods, and future directions. D. A. Andreen^{*1}, E. I. Hansen¹, H. Hu¹, S. P. HekmatiAthar², V. S. Vempalli¹, K. R. Briggs³, G. J. Thoma⁴, and J. P. Oliver¹, ¹Cornell University, Ithaca, NY, ²University of Wisconsin, Madison, WI, ³Fairlife, Chicago, IL, ⁴AgNext, Colorado State University, Fort Collins, CO.

Manure is a critical component of nutrient cycling on dairy farms, but greenhouse gas emissions from manure also represent a considerable fraction of total emissions at the farm and industry level. Quantifying manure emissions is challenging as manure systems can be complicated and variable between farms, and biological and chemical processes underlying emissions are complex. The manure module is a crucial link in modeling circular nutrient flows on farms within the Ruminant

Farm Systems Model (RuFaS). The objective of this symposium session is to review the methods, function, and future directions of the manure module. The main purpose of the manure module is to track the fate of nutrients (e.g., N, P, K, C) from excretion by animals until field application or off-farm export. The module quantifies manure nutrients available for agronomic or other use and illustrates how management choices impact manure composition and emissions. Manure is received daily from the animal module, which predicts manure mass and composition based on animal characteristics and nutrient intake, directly linking on-farm nutrient cycling to animal management. In the manure module, manure moves through a user-defined management chain including options for manure handling, mechanical solid-liquid separation, anaerobic digestion, and storage in liquid, slurry or solid form. Daily nutrient and mass transformations, losses (e.g., volatilization), and gains (e.g., bedding, wash water) are quantified and updated at each step along the chain. Development of the manure module is limited by insufficient data and understanding of the basic processes driving emissions, as well as multisite, applied research quantifying the impact of manure management on these processes. Process-based methods are prioritized for inclusion over empirical methods wherever possible but empirical models and emissions factors are incorporated as needed. In addition to supporting dairy farm systems analysis and relevant industry applications, the RuFaS manure module can serve as a tool to aid in identification of critical knowledge gaps and an avenue for rapid assimilation of new research.

Key Words: manure management, modeling, nutrient tracking

1365 The RuFaS soil and crop module: Integrating extant field-simulation models for flexible representation of dairy cropping systems. P. Adhikari^{*2}, C. Marrow¹, S. P. HekmatiAthar⁴, E. Hansen³, K. Panke-Buisse¹, A. Rawal², E. Young², P. Vadas⁵, and K. F. Reed¹, ¹USDA-ARS, Dairy Forage Research Center, Madison, WI, ²USDA-ARS, Dairy Forage Research Center, Marshfield, WI, ³Cornell University, Ithaca, NY, ⁴University of Wisconsin, Madison, WI, ⁵USDA-ARS, Washington, DC.

Soil and crop is one of the 4 core biophysical modules in the Ruminant Farm Systems (RuFaS) model. This module simulates any number of fields, each with a configurable number of soil layers and options for growing and harvesting 22 common dairy crops from 7 plant species with explicit daily simulation of crop growth, C/N/P/Water Cycles, and erosion on a daily time step. The methodology of the soil and crop module integrates 3 widely used existing field and biogeochemical models (the Soil and Water Assessment Tool, SurPhos, and Daycent) in Python with a flexible field management algorithm that allows the user to specify dates and attributes of fertilizer and manure applications, irrigation, tillage, planting, and harvesting. Simulated outcomes include crop yield, nutrient content, and abiotic stresses such as temperature, moisture, and nutrient availability. In addition, the soil and crop module simulates soil nutrient transformations in response to environmental conditions including 6 soil organic and inorganic N pools, 3 soil phosphorus pools, 4 soil carbon pools, and soil water fluxes throughout the soil profile. Module-predicted crop yields, leaching, runoff, soil carbon sequestration, and emission of nitrous oxide, and ammonia under varying manure and synthetic fertilizer application rates and environmental

conditions align with expected outcomes and model behavior. The soil and crop module receives manure inputs from the manure module in the form of liquid, slurry, or solid manure and reports harvested crops as an output for storage as dry hay, silage, or baleage. User-inputs include soil type and composition characteristics such as, bulk density, texture, moisture, and initial nutrient values, crop type, and field management practices. As a modular component of RuFaS, the soil and crop module represents an essential part of the dairy farm nutrient cycle and will help researchers and dairy producers identify management options for overcoming challenges related to efficiency, profitability, waste, and environmental impacts.

Key Words: nutrient cycling, modeling, cropping systems

1366 The RuFaS feed storage module: Connecting field and feed management to animal performance. K. Panke-Buisse^{*1}, S. P. HekmatiAthar², E. Hansen³, and K. F. Reed¹, ¹USDA-ARS U.S. Dairy Forage Research Center, Madison, WI, ²University of Wisconsin–Madison, Madison, WI, ³Cornell University, Ithaca, NY.

The feed storage module's role in the Ruminant Farm Systems (RuFaS) model is as a dynamic inventory of purchased and farm-grown feed and forage and their nutrient qualities. Feed may be added via purchase through the diet formulation function in the animal module and from the on-farm harvest function of the soil and crop module. Feed is removed from the inventory when it is fed out in the animal module and through loss predictions during storage and feeding. The feed storage module currently accepts corn (silage, dry/high-moisture grain), alfalfa, grass, cereal or small grain, and soybean to be stored dry (hay, grain, and concentrates), ensiled (in bag, bunker, or pile), or as baleage. Stored feed quantity and quality changes are simulated over the duration of storage depending on crop type, crop quality, user-specified storage conditions, and ambient climate conditions. Feed component values, proportions, digestibility, and availability subject to change during storage are mass, DM content, CP, NPN, NDF, ADF, starch, and water-soluble carbohydrates (WSC). Dry matter is lost during storage as gas, effluent, or particulates reduces the mass of feed components accordingly to accurately reflect the remaining feed and its qualities. Forage or feed quality can also be user-specified both before and after storage, offering flexibility and modularity of user needs. Stored feed can facilitate ration formulation in the animal module directly based on its simulated nutrient composition or by assigning a quality rating to align with published feed quality values.

Key Words: feed storage, forage conservation, modeling

1367 Optimizing dairy farm profitability and sustainability with the RuFaS economics module. J. Greene^{*1}, B. Limb¹, J. C. Waddell², and P. Hekmati³, ¹Sustainability Science, Steamboat Springs, CO, ²Dairy Management Inc., Rosemont, IL, ³University of Wisconsin, Madison, WI.

Economic considerations are a key driver of sustainability in dairy production, shaping the adoption and abandonment of diverse farm management practices and technologies. The Ruminant Farm Systems (RuFaS) model provides comprehensive environmental analysis for dairy operations; however, a robust economic module is essential for evaluating trade-offs between environmental and financial performance. This will be achieved by the RuFaS economics module, designed to integrate farm-scale financial modeling with scenario-based decision support. Although still under development, this presentation will provide

an overview of its design structure, modeling approaches, key functionalities, and application for optimizing farm profitability and sustainability. The economics module connects farm management decisions to key financial metrics, integrating capital expenditures, operational costs, revenue streams, and financing structures. Designed for flexibility, it facilitates comprehensive assessments of return on investment, payback periods, and cost-benefit analyses across diverse dairy interventions. Key capabilities include evaluating the economic feasibility of anaerobic digesters by comparing revenue from biogas and carbon credits against capital investment, assessing trade-offs in feed production versus purchase decisions, and analyzing the financial impact of regenerative practices such as no-till farming and cover crops. The module is being developed through iterative testing with subject matter experts and will integrate with the existing RuFaS biophysical and energy use modules. Expected outputs include scenario-driven financial projections, sensitivity analyses, and integration with environmental performance metrics to provide holistic sustainability insights. By equipping farmers and advisors with transparent, data-driven economic assessments, the RuFaS economics module aims to accelerate the adoption of financially viable, sustainable dairy farming practices.

Key Words: dairy economics, whole-farm modeling, integrated assessment model

1368 Farmers Assuring Responsible Management Environmental Stewardship program integrates the Ruminant Farm System model. N. Ayache^{*1}, S. Saffran¹, J. S. Adamchick², J. C. Waddell³, S. P. HekmatiAthar⁴, and K. R. Briggs³, ¹National Milk Producers Federation, Arlington, VA, ²Cornell University, College of Veterinary Medicine, Ithaca, NY, ³Dairy Management Inc., Rosemont, IL, ⁴University of Wisconsin–Madison, Madison, WI.

The National Milk Producers Federation (NMPF), in partnership with Dairy Management Inc., created the National Dairy Farmers Assuring Responsible Management (FARM) Program in 2009 as a customer and consumer assurance program related to on-farm social responsibility, paid for and governed by US dairy farmers. In 2017, the program added the FARM Environmental Stewardship (ES) pillar to provide a comprehensive estimate of the GHG emissions and energy use on dairy farms. The FARM program undergoes a cycle of review and updates every 3 yr. In its Version 3.0 update in 2024, FARM ES integrated RuFaS to become the first process-based model for greenhouse gas accounting available to US dairy farmers. Processors and cooperatives representing 80% of the US milk supply currently participate in FARM ES and a working group of industry representatives including field staff and farmers contributed to the piloting and integration efforts. To evaluate the capacity of the RuFaS model to represent diverse dairy animal and manure management systems, we collected information on management practices to inform model inputs from 32 volunteer farms across the US and applied the RuFaS model to estimate the GHG emissions from animals (enteric CH₄), manure (CH₄, direct N₂O, and indirect N₂O), and feed production (CO₂-eq). Volunteer farms varied in geographic location, herd size (30 to 10,000), herd management practices, and manure management strategies. Feedback from the volunteer pilot farms, co-op and processor field staff, and the FARM ES Working Group was used to improve the alignment between RuFaS inputs and on-farm management practices as well as enhance practice inclusion, reporting, and the user interface. FARM ES Version 3.0 with the integration of RuFaS has now been launched for use by US dairy producers, cooperatives, and processors. Due to the process-based nature of RuFaS, FARM ES now enables retrospective analysis of a farm's footprint and comparative

“what-if” scenario analysis to support on farm decision making and advance US dairy’s efforts toward GHG neutrality.

Key Words: participatory modeling, greenhouse gases, environmental impact

1369 RuFaS as an open-source platform: What does it mean for your science program? J. C. Waddell^{*1}, S. P. HekmatiAthar², J. M. Tricarico¹, and K. F. Reed³, ¹*Dairy Management Inc., Rosemont, IL*, ²*University of Wisconsin, Madison, WI*, ³*USDA-ARS, Madison, WI*.

The Ruminant Farm Systems model (RuFaS) is an open source, process-based model, and this presentation explores how this platform improves access to information and advances opportunities for collaboration. Commitment to open-source means that users can explore every line of code in the simulation and fully trace the methods applied to produce any given output for every simulation. A strength of the open-source approach is that, by taking advantage of the virtual repository and version control platform GitHub, anyone that adheres to the requisite processes outlined in RuFaS’s charter can directly contribute to model development. Following the established protocols, anyone can report issues, request new features, and provide feedback on any part of RuFaS via the

online repository. Git version control also allows users to easily make their own “branches,” or copies of the model, to make modifications for testing and evaluating new methods without affecting the current main version of RuFaS. Model modifications can be incorporated into the main branch after completing a review process. All potential contributions to the codebase, whether they are from current development team members or new contributors, can be submitted to be considered for integration into the main version of RuFaS through a thorough evaluation, testing, and documentation process. All interactions with the RuFaS codebase are documented and archived, allowing users to see details as to why specific changes were implemented or rejected. Several enhancements to the RuFaS model are in various states of development, including an animal health submodule, grazing systems, and water quality assessment. Given the rapidly changing landscape in the dairy sector, an adaptable system is necessary for long-term utility, and this is intrinsic to the architecture of the software. By using modern software engineering principles—which ensure that existing code facilitates improvement and expansion in the areas most relevant to the dairy industry—RuFaS provides a platform for scientific collaboration and not “just” a dairy farm model.

Key Words: modeling, environment, production

Ruminant Nutrition Symposium: Joint ADSA-NANP-ASAS-PSA Session— Anomalies in Analyzed Nutrient Composition of Feedstuffs

1370 Unassigned organic matter in feeds. F. N. Owens^{*1}, B. B. Grimes Francis², and Z. K. Smith², ¹Oklahoma State University, Stillwater, OK, ²South Dakota State University, Brookings, SD.

Proximate analysis of feedstuffs is comprised of determination of 5 nutrients: moisture, crude ash, CP, extractable fat, and crude fiber. Nitrogen-free extract (NFE) is determined as the difference between the sum of these nutrients and DM. In tabular feed composition values, each of the 6 nutrients are routinely expressed as a fraction of feed DM. Yet, the sum of the individual nutrients often totals less than 100% of the DM portion. This difference or “undefined fraction” is denoted as residual organic matter (ROM) from the equation: $ROM = 100 - \text{ash} - CP - NDF - \text{starch} - (FA/FatFactor) - (CP - 0.64 \times sNPNCPE)$ and is assumed to have an energy value of 4 Mcal/kg. This deficit fraction is substantial for some feedstuffs (11.2% for corn grain, 8.35% for corn silage, 51.4% for wet citrus pulp, -3.05% for feather meal). Therefore, the question is posed, why doesn't the sum of its parts on a DM basis not equal the whole? Components of fermented feedstuffs may be lost during oven drying as volatile substances, minerals may be lost during oven-drying by converting anions to carbonate, thereby falsely increasing ash weight. Formation of Maillard products during drying, and under-recovery of ROM also occurs whenever feed components are not properly dried or assayed, particularly the nonstructural non-starch carbohydrates (NSNSC), pectins, and β -glucans. Certain nutrients may also be present in multiple fractions (e.g., ash and CP [$N \times 6.25$]). Therefore, correction of NDF for protein and ash would seem preferable. It is recommended to include mean values for ROM and NSNSC within tabular feed tables as an index of the extent of unidentified substances and thereby the degree that energy values and digestibility may be altered to compensate for missing or excess DM or OM. Nutritional values of feedstuffs are imperative to diet formulation and discrepancies in actual versus estimated values may be underestimated and pose significant performance and economic detriments.

Key Words: feed analysis, nonstructural nonstarch carbohydrate, organic matter

1371 Inconsistencies in fiber analysis affecting feed analysis databases. D. R. Mertens^{*}, *Mertens Innovation & Research LLC, Belleville, WI.*

Fiber is a nutritional entity that is defined by the method of measurement. Methods of drying or grinding samples, reagents or extraction methods, filtration methods or media, and basis for reporting results have changed over time. When created in the 1960s, NDF used sodium sulfite to reduce protein contamination. In the 1970s, starch contamination was discovered as a problem for analyzing corn silage and grains and the method was revised to include heat-stable α -amylase, but sulfite was removed because it might reduce lignin. Sometimes this NDF was adjusted for protein contamination. In the 1990s, the AOAC Official Method 2002.04 for aNDF was developed to include both sulfite and amylase, with an option for ash-free aNDF. This method included modifications that can alter results for specific feeds that are finely ground or contain excessive fats, pectins, or mucilages. Currently, ash-free NDF is the preferred method, but before this NDF was not measured as ash-free. The AOAC method for ADF included lignin analysis using sulfuric acid and asbestos as a filter aid. Banning of asbestos required modifications of the original

lignin method and a permanganate method for lignin was developed, which makes reporting of ADL ambiguous. All of these methods were based on cutter-mill grinding through 1-mm screens and collection of fiber on cindered glass crucibles, with or without filter aids. Filter bag methods were developed for NDF, ADF, and lignin, that involved multiple updates in both methodology and filter bags, sometimes caused inconsistencies. Currently, most fiber results are generated by NIRS, which may introduce variability due to differences in calibration datasets and approaches. Finer grinds of NIRS samples will result in lower chemical analyses of fiber, when not using filter aids. Changes in methods may result in less than ± 5 percentage-point changes in fiber values from published historical sources. Nevertheless, fiber analyses have proven useful in evaluating feeds and formulating rations. Simple averages of large numbers of unedited results from unknown methods, which are not internally consistent with themselves or other analyses, may be neither informative nor useful.

Key Words: NDF, ADF, lignin

1372 Protein analysis methodology. G. A. Broderick^{*}, *US Dairy Forage Research Center, Madison, WI.*

Crude protein has been estimated since the mid-19th century as $N \times 6.25$. Dependence on Kjeldahl assays for feed N has been largely replaced by safer and more precise elemental N analyses; however, elemental analyzers also detect nitrate-N as part of total N. Total feed N itself says little about protein utilization. In dairy cattle nutrition, we must quantify feed contribution to RDP and RUP, plus we need to know the EAA composition of RUP digested in the small intestine. Feed RDP and RUP values are largely estimated indirectly using rates of CP disappearance from in situ bags; assumed rumen passage rates, and proportions of feed protein degraded and escaping the rumen are computed applying simple exponential models. In situ techniques have proven useful for more than 50 yr, but the assay has at least 3 inherent flaws: (1) solubility affects rate of CP disappearance and not all soluble proteins are equally degraded; (2) microbial CP contamination of in situ residues underestimates degradation, particularly for low CP feeds; and (3) nutritional value of feed RUP is largely based on digestibility and EAA analyses of feed before the in situ phase. Intestinal RUP digestibility can be estimated by a “3-step assay” where in situ residues from a single in situ incubation are subjected to proteases mimicking in vivo abomasal and intestinal digestion; however, both degradation and passage rates influence the correct incubation time. Published in situ RDP/RUP values for certain feeds proved unreliable when fit into the NASEM protein model, requiring alteration of rumen passage rates. Determination of feed EAA content requires acid hydrolysis plus basic hydrolysis for Trp; recently, mathematical adjustments were used to correct for loss or incomplete release of AA during hydrolysis. Modern analytical methods have reduced analysis time and improved accuracy of AA determination; NIRS calibrations for EAA quantitation are being developed. The NASEM model estimates milk protein yield from supply of several EAA not just that first-limiting, thus supply of all EAA should be known.

Key Words: feed protein analysis, rumen undegraded protein (RUP), EAA composition

1373 Success and continuing challenges in analyzing nonfiber carbohydrates. M. B. Hall*, *US Dairy Forage Research Center, USDA-ARS, Madison, WI.*

Analysis of non-NDF carbohydrates (NFC) and their use in dairy cattle nutrition has progressed as we've partitioned a by-difference fraction into more nutritionally relevant fractions, but challenges remain. Currently, measured fractions include starch and water-soluble carbohydrates (WSC), and there is a calculated by-difference residual fraction (BDRF) that encompasses pectins, mixed-linkage β -glucans, and other unmeasured NFC. Starch analysis on gelatinized samples defines starch as glucose released by the hydrolytic action of thermostable α -amylase and amyloglucosidase times 0.9 to account for the weight of water added by hydrolysis. Analyses run at neutral pH give reduced values; failure to account for free glucose in samples inflates values. Without use of alkali or other chemical agent for gelatinization, only non-resistant starch is measured. The WSC encompass mono-, di-, and oligo-saccharides, and some polysaccharides such as fructans. In this empirical assay, samples are extracted with warm water, the sample centrifuged or filtered to remove particulate matter, and the extract analyzed using the phenol-sulfuric acid assay, typically using sucrose as the standard. Because different standards (e.g., glucose, lactose, sucrose) give different standard curves, use the likely predominant WSC in a sample as the standard for that sample. Because WSC will have some compositional diversity in most if not all samples and are measured against 1 standard, the WSC value will be an estimate. Water-soluble starch is detected as both starch and WSC. The WSC extract can be analyzed for free and enzymatically released glucose, allowing correction of the starch value for soluble starch. Long chain fructans and lactose are measured in WSC, but are not soluble or measured if 80% ethanol is the extractant. The BDRF is problematic. It is comprised of carbohydrates and unmeasured non-carbohydrates, and is affected by nitrogen factors and analytical error; the variance of BDRF values equals the sum of the variances of the analyses used to calculate them. Approach calculation and nutritional interpretation of BDRF with caution.

Key Words: starch, nonfiber carbohydrate, analysis

1374 Assessing accuracy and validity of assays from commercial feed analysis laboratories. N. Schlau*, *Dairyland Laboratories, Arcadia, WI.*

Accurate and consistent analysis of feedstuffs is fundamental to supporting livestock nutrition, ration formulation, and research. However, multiple sources of error can obscure true compositional differences and introduce uncertainty into feed evaluation. Analytical error begins at collection, where factors such as inadequate sampling techniques and improper handling affect results before the sample even reaches the laboratory. Classification and sample preparation introduce further opportunities for error (e.g., misidentification or contamination). Near-infrared reflective spectroscopy (NIR) has become essential for ration formulation because it is a rapid and economical analysis. However, traditional NIR calibrations depend on pre-identification of sample types. Misidentification significantly reduces accuracy of predictions,

and statistical tools for detecting such samples are imprecise. Contamination can occur during collection, sub-sampling, or processing, also impacting accuracy and interpretation of results. To mitigate these challenges, it is crucial that commercial feed laboratories implement quality assurance measures to ensure accuracy and validity of results. Many nutritional analyses are empirical measurements; the result is not a specific ion or molecule but is defined by the assay used to measure it. This makes quality control particularly challenging because we cannot rely on known chemical standards. Instead, systemic error is monitored through participation in certification programs and occasional round robin testing to assess whether there are consistent biases in a particular analysis. Internally, we use control charts to track trends and detect drifts requiring correction. Individual sample errors must also be identified, which are addressed through plausibility checks, including comparisons to historical data and expected ranges. The presentation concludes with a discussion on interpreting outlier results, providing examples of how a commercial laboratory differentiates analytical errors stemming from technical issues versus true outliers that challenge established nutritional assumptions.

Key Words: feed analysis, nutrient composition, analytical variability

1375 Development of an evergreen, interactive, and consolidated dataset of feedstuff nutrient composition. R. N. Dilger*, *University of Illinois at Urbana-Champaign, Urbana, IL.*

The objective of this presentation is to highlight how the National Animal Nutrition Program (NANP) Feed Composition Database addresses persistent challenges in feed analysis by providing a continuously updated, standardized, and publicly accessible dataset. Accurate characterization of feedstuffs is essential for formulating balanced diets, optimizing animal performance, and ensuring economic and environmental sustainability in livestock production. However, unexplained variation in nutrient composition, methodological inconsistencies, and gaps in available data complicate precise nutrient delivery. The NANP Feed Composition Database is designed to mitigate these challenges by consolidating compositional data from a wide variety of feedstuffs, enabling more accurate predictions of nutrient availability and utilization as part of the feed formulation workflow. This dynamic resource integrates historical and contemporary data, ensuring flexibility to incorporate emerging analytical methods while maintaining a consistent framework for diet formulation and modeling applications. By synthesizing diverse data sources, the database serves as a cornerstone for advancing nutritional science, enhancing the precision of nutrient requirement models, and improving decision making in feed formulation across production systems. As analytical techniques evolve and new feed ingredients emerge, a centralized, evergreen database is essential for supporting nutritionists across species. The NANP Feed Composition Database provides the foundation needed to bridge knowledge gaps in nutrient composition research, improve data harmonization, and enhance the practical application of feed analysis in animal nutrition.

Key Words: National Animal Nutrition Program, feed composition database, nutrient variability

Ruminant Nutrition 3

1376 Effect of a specific magnesium oxide blend on ruminal pH and milk production in dairy cows: A meta-analysis. R. Balegi¹, M. Baudon¹, A. Courillon¹, J. Le Cour Grandmaison¹, and A. Bach^{2,3}, ¹Timab Magnesium, Saint-Malo, Illes et Vilaine, France, ²University of Lleida, Lleida, Catalonia, Spain, ³Institució de Recerca i Estudis Avançats, Barcelona, Catalonia, Spain.

Data were compiled from 2 independent datasets, derived from studies each lasting 3 mo, to run a meta-analysis to evaluate the effects of a specific magnesium oxide blend (MG) supplementation on ruminal pH stability and milk production in dairy cows. Treatments compared MG (85 g/cow per day; on average) to other buffering agents (OTHER: sodium bicarbonate, lithothamnium, and MgO with sodium bicarbonate, all formulated to have a similar neutralizing capacity). One dataset included 8 studies in 84 cows (41 MG, 43 OTHER) equipped with ruminal boluses recording pH every 10 min. Measured parameters included mean, minimum, and maximum pH, time spent in acidosis (pH <5.8 and pH <6.0) and in optimal conditions (6.2 < pH < 6.5), and hourly pH evolution. The other one consisted of 7 experiments evaluating weekly milk production and composition (fat and protein) in 808 cows (378 MG, 430 OTHER). Days in milk averaged 156 d for MG and 152 d for OTHER, with an average lactation number of 2.9 and 2.8, respectively. Treatment effects on ruminal pH variables and milking performance were analyzed using a mixed-effects linear model with treatment as fixed effect, and study and cow nested within study as random effects. Lactation number and DIM were included as fixed effects. Hourly pH was analyzed similarly, including hour and its interaction with treatment as fixed effects. Cows on MG spent less time at pH <5.8 (54.5 vs. 139 min/d, $P = 0.016$) and pH <6 (180 vs. 311 min/d, $P = 0.018$) while tending to spend more time (+81 min/d, $P = 0.103$) in the optimal range relative to OTHER. Cows on MG had greater minimum pH (6.04 vs. 5.93, $P = 0.034$) and maintained a consistently higher pH ($P < 0.001$) throughout the day compared with OTHER. Milk yield tended to be higher with MG (+0.91 kg/cow per day, $P = 0.102$), whereas milk fat (3.99% vs. 4.02%) and protein (3.38% vs. 3.40%) remained unchanged relative to OTHER.

Key Words: buffering agent, rumen acidosis, ruminal pH

1377 Two different strategies but one result: Live yeast and yeast postbiotics have similar effects on dairy cow mid-lactation performance. W. L. S. Reis*, G. A. C. Valini, and M. Bonato, *ICC Animal Nutrition, Research and Development, Jundiaí, SP, Brazil.*

Yeast supplements are usually added to dairy feeds to improve the ruminal environment, which may positively influence cows' milk production. Live yeast (LY) is known to create a more anaerobic environment for the host microbiota, while yeast postbiotics (YP) compounds have been reported to improve nutrient digestibility. Despite those elicited effects, questions about whether both strategies would impair milk production and quality to the same magnitude. Therefore, this study aimed to evaluate the impact of LY or YP supplementation on dairy cow performance. Cows ($n = 44$) were blocked according to parity and milk yield and randomly assigned to a diet with 5 g of LY (Actisaf) or 14 g/d YP (RY40) for 6 wk during mid lactation. The nutrient intake and milk performance were analyzed using F test in GenStat. After 6 wk of trial, treatments did not influence DMI, efficiency, milk composition, or SCC ($P > 0.05$). However, YP had a higher BW ($P < 0.01$) and BCS ($P = 0.03$) compared with LY (Table 1). The YP may positively affect

available glucogenic nutrients in the rumen, which may have resulted in a positive energy balance and higher BW and BCS. Supplementing LY or YP results in similar mid-lactation performance but with a positive energy balance in YP-fed cows.

Key Words: *Saccharomyces cerevisiae*, milk production

1378 Supplementation of hydrogenated fat-embedded calcium gluconate improves apparent total-tract digestibility in lactating Holstein-Friesian cattle. D. J. Seymour*, M. V. Sanz-Fernandez, J. B. Daniel, and J. Martín-Tereso, *Trouw Nutrition R&D, Boxmeer, the Netherlands.*

Hydrogenated fat-embedded calcium gluconate (HFCG) has been shown to increase milk fat or protein yields in lactating dairy cattle. Although the mode of action remains unclear, previous work has shown changes in microbial composition and VFA concentrations that confirm HFCG fermentation in the lower digestive tract. However, how changes in the hindgut ultimately translate into performance improvements is currently unknown. The objectives of this study were to evaluate production and apparent total-tract digestibility (ATTD) in response to HFCG supplementation in a dose-dependent manner (0%, 0.05%, 0.09%, and 0.18% of diet DM). Forty-eight lactating Holstein-Friesian dairy cattle (16 primiparous, 32 multiparous) were used in a 4×4 replicated Latin square. Cattle were blocked by parity, stage of lactation, and milk yield, and randomly assigned 1 of 4 treatment sequences. Within periods, animals were adapted to treatment TMR for 14 d. Feed intake, milk, and component production were recorded from d 15 to 21 of each period, with fecal grab samples collected twice daily on d 15, 17, and 19. Using TiO_2 as external marker, ATTD was determined on a subset of cattle ($n = 5$ per treatment sequence; 20 cattle total). Averages of data collected during the final week of each period were analyzed considering a parity

Table 1 (Abstr. 1377). Nutrient intake, efficiency, milk yield, composition, BW, and BCS in dairy cows fed live yeast (LY) or yeast postbiotics (YP) in mid lactation

Item	Treatment		SEM	P-value ¹
	LY	YP		
DMI (kg/d)	23.7	23.5	0.19	0.65
Basal ration (kg DM/d)	16.4	16.2	0.25	0.75
Concentrate (kg DM/d)	7.3	7.3	0.05	0.76
TMP intake ² (g/d)	2,102	2,092	14.8	0.62
Feed efficiency ³ (%)	1.31	1.31	0.02	0.98
N-efficiency ⁴ (%)	27.6	27.4	0.35	0.56
Milk (kg/d)	29.8	29.6	0.40	0.65
Milk fat (%)	4.23	4.18	0.07	0.65
Milk protein (%)	3.76	3.77	0.02	0.74
Lactose (%)	4.53	4.54	0.02	0.62
Milk urea (mg/dL)	22.7	22.4	0.39	0.61
SCC (log)	1.73	1.73	0.04	0.96
BW (kg)	732	738	1.43	<0.01
BCS	3.37	3.42	0.02	0.03

¹Bolded values indicate statistical significance at $P < 0.05$.

²TMP = total metabolized protein.

³Feed efficiency = kg fat- and protein-corrected milk/kg DMI.

⁴N-efficiency = milk N/N intake.

group-specific intercept (primiparous or multiparous) and HFCG dose up to a cubic term as fixed, and the random effects of cow and period. Milk protein yield increased linearly with HFCG dose (162 g/d per % HFCG inclusion; $P = 0.025$), while milk urea displayed a quadratic response ($P = 0.022$) with a maximal response of 0.93 mg/dL greater than the negative control ($P = 0.016$) at a HFCG dose of 0.11% DM. In response to HFCG dose, ATTD of DM, NDF, ADF, CP, starch, OM, and gross energy all increased linearly ($P \leq 0.008$), with slopes of 13.5% (DM), 23.1% (NDF), 24.4% (ADF), 14.7% (CP), 2.58% (starch), 12.3% (OM), and 12.9% (gross energy) per % HFCG inclusion. These results suggest that the performance benefits often observed when supplementing HFCG to lactating dairy cows may be partially explained by improvements in digestibility.

Key Words: prebiotic, hindgut, gut health

1379 Effect of the timing of ruminal infusion of sodium bicarbonate on nutrient digestibility and rumen metabolites analyzed by NMR in dairy cows. M. Arif*, F. Hao, C. Matamoros, A. Patterson, and K. J. Harvatine, *Pennsylvania State University, University Park, PA.*

Rumination is very important to rumen mixing, physical reduction of particle size, and addition of salivary buffers. There is a robust daily pattern of rumination with less rumination during the daytime and more during the night. The objective of the study was to mechanistically explore the impact of the daily rhythm of buffer addition to the rumen on nutrient digestibility and rumen environment. Ten ruminally cannulated Holstein cows (5 primiparous and 5 multiparous) were used in a 4×4 Latin square design with 16 d rumen infusion periods and a 5-d washout between periods. Cows were fed the same corn-silage-based TMR and were ruminally infused with either 10 L of water or 10 L of a solution containing 500 g of NaHCO_3 . Treatments were infusion of NaHCO_3 either during the day (DAY; 0800 to 1700 h), during the night (NGT; 2000 to 0500 h), or during both the day and night (D+N; 0800 to 1700 h and 2000 to 0500 h), or infusion of water across the day and night (CON; 0800 to 1700 h and 2000 to 0500 h). Data were analyzed using a mixed model that included the random effect of cow and period and the fixed effect of treatment for nutrient digestibility and repeated measures were used for rumen metabolites. Daily intake and total-tract apparent digestibility of DM, OM, NDF, and FA were not affected by treatment ($P > 0.05$). We quantified 33 rumen metabolites by nuclear magnetic resonance. Succinate was decreased by DAY, NGT, and D+N compared with CON ($P < 0.05$). A treatment-by-time interaction was observed for xanthine ($P = 0.03$), where it was decreased by DAY except at 2000 h and decreased by NGT during the early morning (0400 to 0800 h) compared with CON. Although 24 out of the 33 rumen metabolites were affected by time ($P < 0.05$), they were not affected by treatment nor had treatment by time interactions. Overall, nutrient digestibility, and rumen metabolites other than succinate and xanthine were not affected by the timing of NaHCO_3 infusion.

Key Words: nuclear magnetic resonance, fermentation, buffer

1380 Effects of reducing dietary sodium by removing sodium chloride or sodium bicarbonate on production and nutrient digestibility in lactating cows. D. Espinoza^{1,2}, S. Majeesh², C. Lee³, and Y. Ben-Meir^{*1}, ¹*Department of Ruminants Science, Agricultural Research Organization – Volcani Institute, Rishon LeZion, Israel*, ²*Department of Animal Science, The Hebrew University of Jerusalem, Rehovot, Israel*, ³*Department of Animal Sciences, The Ohio State University, Wooster, OH.*

Excess dietary Na results in increased Na excretion in manure, which can damage soil and groundwater quality. We reduced dietary Na by removing NaCl or NaHCO_3 . Accordingly, the following dietary treatments were investigated: (1) CON (0.465% Na in dietary DM with added NaHCO_3 and NaCl); (2) NHC (0.306% Na with added NaHCO_3); and (3) NC (0.306% Na with added NaCl). Forty-two multiparous mid-lactating cows were divided into groups of 3 according to days from calving, BW, and yield, and randomly assigned to one of the above listed treatments. The experiment was conducted with 2 wk of diet adaptation followed by 7 wk of sampling during which individual feed intake, milk and milk components yield, and BW were measured. During the last 5 d feces, urine, rumen fluid, and milk samples were collected. For 4 cows of each treatment, we inserted boluses (Moonsyst, Ireland) into the reticulorumen for continuous measurements of rumen pH. Data were analyzed using the mixed-model procedure in JMP Pro 16 with treatment, day, and treatment $\times$ day as fixed effects, cow as random effect, and daily data as repeated measures modeled as AR(1). Reducing NaHCO_3 or NaCl did not affect DMI (30.4 kg/d) or ECM yield (41.5 kg/d). Total-tract digestibility of DM, OM, N, and NDF were not affected by treatments. Continuous rumen pH measurement revealed that rumen pH was higher ($P < 0.01$) for NHC and NC (6.40 and 6.41, respectively) compared with CON (6.11). In conclusion, reducing dietary Na to 0.306% did not affect production of cows and, therefore, have potential to reduce the environmental impact caused by Na excretion. Reducing dietary Na maintained relatively high rumen pH during the day and night, warranting further investigation of the role of Na on rumen activity.

Key Words: rumen pH, buffer, Israeli Holstein

1381 Using collar-based algorithm to estimate individual feed intake and efficiency in commercial farms. R. Yair*, G. Katz, A. Arazi, and V. Shabtai, *Afimilk, Kibbutz Afikim, Israel.*

Feed efficiency (FE) is an important trait, because it is established as hereditary and directly affects profitability and sustainability. A limiting factor to individual FE determination in commercial farms is the need for individual DMI. Most DMI equations use data unavailable to many commercial farms and can be inaccurate in cows with relatively high or low DMI. Integrating eating behavior parameters from wearable sensors, may enable more accurate and reliable DMI prediction. Therefore, the objectives of this work were to develop an algorithm to accurately predict individual DMI based on Aficollar neck-tag and milk data, and examine the potential of using FE, calculated with predicted DMI, to improve herd efficiency. Accordingly, weekly DMI data from 3 research farms in the United States, Canada, and Italy (100, 100, and 50 cows wearing Aficollar neck-tags, respectively) were received. Aficollar data describing eating, rumination and panting behavior were processed and combined with basic cow data (DIM, lactation, pregnancy status) and milk production. Machine learning methods were used to produce a model predicting weekly DMI, which was trained on one research farm data (100 cows, 4,500 weekly data points) and validated on the other 2 (150 cows, 6,000 weeks). Statistical analysis was done in Python using ANOVA and Pearson correlation. Dry matter intake predictions of the 2 validation farms were compared with the measured DMI, R^2 was 0.78, and RMSE was 1.82 kg. To examine the heritability of FE, defined as ECM/predicted DMI, data were collected from 5 Israeli farms. Average FE of each cow between 100 to 200 DIM was calculated. The FE of dams ($n = 280$) was correlated with that of their offspring ($n = 340$), R^2 was 0.27 ($P < 0.0001$). Feed efficiency from 145 offspring of 25 bulls with at least 5 offspring was collected, and the bull effect was determined significant using a F-test ($P = 0.01$). These results demonstrate that a collar-based algorithm can accurately predict individual DMI. Moreover,

the relationship between parental and offspring FE suggests that using FE data to support breeding decisions should improve offspring FE.

Key Words: feed efficiency, collar, dry matter intake

1382 Variations in blood amino acids and acylcarnitines in early lactating Holstein-Friesian crossbreds differing in milk yield in smallholder dairy farms. A. Tadesse*^{1,2}, M. Birhan³, Y. Mekuriaw⁴, P. Lutakome^{1,5}, D. Asizua⁶, D. N. Khang⁷, Y. Tesfay⁸, L. D. Phung⁹, and V. Fievez¹, ¹Ghent University, Ghent, Belgium, ²Mekelle University, Mekelle, Ethiopia, ³University of Gondar, Gondar, Ethiopia, ⁴Bahir Dar University, Bahir Dar, Ethiopia, ⁵Makerere University, Kampala, Uganda, ⁶National Livestock Resources Research Institute, Entebbe, Uganda, ⁷Nong Lam University, Ho Chi Minh, Vietnam, ⁸Independent consultant, Mekelle, Ethiopia, ⁹Hue University, Hue, Vietnam.

The study aimed to assess variations in dried blood spot (DBS) free amino acids (AA) and acylcarnitine (ACC) profiles among crossbred cows managed in Ethiopian smallholder dairy farms, clustered based on their milk yield. The DBS were sampled on d 3, 6, 9, 21, and 90 postpartum from 52 multiparous Holstein-Friesian crossbred cows. K-means clustering grouped cows based on average milk yield on d 60 and 90 into low (4.25–7.2 L/d), medium (7.3–12.2 L/d), and high (12.3–20 L/d) milk yield clusters. The DMI of cows in the high milk yield cluster (HMYC) and low milk yield cluster (LMYC) was 9.7 and 8.2 kg/d, respectively. Cows in the HMYC had elevated dietary NEL and CP content compared with cows in the LMYC. Nevertheless, theoretical fulfillment of the CP and NEL requirements was only 82% and 86%, respectively, for the HMYC cows, whereas intakes recorded for cows of the LMYC adequately satisfied both total CP and NEL requirements. In all groups, diets were unbalanced with CP as the limiting nutrient for milk production. Cows of the HMYC had higher concentrations (μmol/L) of glycine, ornithine/citrulline, and NEAA, but lower concentrations of carnitine (C0), C5, total long chain acylcarnitines, and the ratio of C16 and C18 to C2 (indicative of carnitine palmitoyltransferase II activity) compared with LMYC cows ($P < 0.05$). The differences between the milk yield clusters in some DBS AA and ACC were as physiologically expected (e.g., reflecting variation in the nutritional status as well as in body fat or protein mobilization). The unexpected elevation of long-chain acylcarnitines (LCAC) levels in LMYC cows, despite a theoretically positive energy balance, suggests incomplete fatty acid β-oxidation. The suggested enhanced lipolysis, indicated by elevated LCAC levels, does not seem to be driven by increased milk production and could potentially be linked to subclinical disorders or

nutrient diversion for other essential physiological functions, warranting further investigation.

Key Words: crude protein, energy balance, dried blood spot, acylcarnitines

1383 Effect of feeding whey permeate on production and rumen conditions in lactating Holstein dairy cows. M. Buckler*¹, R. Gervais², R. Petri³, and J. C. Plaizier¹, ¹University of Manitoba, ²University of Laval, ³AAFC Sherbrooke.

Feeding whey permeate (WP) to dairy cows will reduce surpluses of solids nonfat in the Canadian dairy industry. Effects of including dry WP at inclusion rates of 0%, 4%, 8%, and 12% DM by replacing barley starch were determined in 12 lactating Holstein dairy cows. A 4×4 Latin square arrangement of treatments with 3-wk experimental periods was used, with the final week for sampling and data collection. Rumen fluid was collected by stomach tube before feed delivery and the reticulorumen pH was measured continuously using in-dwelling probes. Body weights and BCS averaged 648 ± 86.9 kg and 2.97 ± 0.35 on a scale of 1 to 5, respectively. Diets were iso-energetic and iso-nitrogenous and averaged 1.67 Mcal/kg NEL and 15.5% CP (DM basis). The WP inclusion did not cause DMI, milk yield, milk protein content, and MUN to differ (Table 1). However, increasing dietary WP increased the contents of fat and BHB in milk. Increasing dietary WP content decreased ruminal concentrations of propionate and increased butyrate, without affecting the concentrations of total VFA, acetate, and ammonia-nitrogen (NH₃N). Diets including WP increased contents of fat and BHB in milk, and butyrate in the rumen, without affecting ruminal pH. The increase in milk fat may be explained by increases in BHB and butyrate, which are precursors for milk fat synthesis.

Key Words: whey permeate, milk composition, beta-hydroxybutyrate

1384 Effects of feeding a *Bacillus subtilis* PB6 product on productive performance, metabolic, and immunological responses during an intramammary lipopolysaccharide challenge on mid-lactation cows. O. Reyes*¹, H. L. Reisinger¹, I. Brown-Crowder², J. E. Hergenreder², L. K. Mamedova¹, T. H. Swartz³, and B. J. Bradford¹, ¹Michigan State University, East Lansing, MI, ²Kemin Industries, Des Moines, IA, ³South Dakota State University, Brookings, SD.

Immunological crosstalk among mucosal tissues is an area of active research, raising questions about the potential for dietary interventions

Table 1 (Abstr. 1383). Results from the inclusion of whey permeate to lactating cow diets

Item	Treatment (% DM WP inclusion)				SEM	Significance (<i>P</i> -values) of contrasts		
	0	4	8	12		Linear	Quadratic	Cubic
DMI (kg/d)	25.4	26.6	26.4	25.9	0.49	0.36	0.03	0.50
Milk yield, kg/d	35.6	36.3	35.5	34.4	0.94	0.17	0.19	0.68
Fat, %	3.88	4.27	4.23	4.44	0.09	<0.001	<0.001	<0.01
Protein, %	3.31	3.32	3.26	3.31	0.03	0.10	0.17	0.46
MUN, mg/dL	11.8	11.9	12.0	12.4	0.44	0.90	0.25	0.10
Milk BHB, mmol/L	0.06	0.08	0.09	0.10	0.009	0.03	0.02	0.002
Ruminal pH	6.4	6.41	6.39	6.41	0.03	0.78	0.7	0.83
Total rumen VFA, mmol/L	75.8	83.0	77.5	81.5	5.52	0.7	0.16	0.9
Rumen acetate, mmol/L	46.7	51.9	48.1	50.7	3.59	0.65	0.14	0.88
Rumen propionate, mmol/L	15.2	15.3	12.7	12.1	1.14	0.25	0.79	<0.01
Rumen butyrate, mmol/L	7.8	9.4	10.0	11.5	1.00	0.17	0.05	0.02
Rumen NH ₃ N, mg/dL	6.26	4.70	5.25	4.73	0.60	0.12	0.02	0.64

to modulate mastitis responses. Our objective was to determine whether a direct-fed microbial would affect circulating immune cell populations or productivity in healthy dairy cows and whether it would modulate physiological responses to an intramammary LPS challenge. Twenty mid-lactation Holstein cows were blocked by parity and DIM, then randomized to treatment sequence in a crossover design with 2 treatments: control (CON; top-dress of 13 g/d limestone) and *Bacillus subtilis* (STAT; PB6 strain, CLOSTAT, Kemin Industries Inc., Des Moines, IA; top-dress of 13 g/d). Periods were 28 d, with an LPS challenge (10 µg in 10 mL of saline, one rear quarter) on d 24 of each period. Milk samples were collected from all 3 milkings on 2 d/wk before challenge. Vaginal temperature was measured every 5 min from d 23 to 27 of each period. Data were analyzed using generalized linear mixed models that included fixed effects of treatment, period, time within period, and 2- and 3-way interactions, with random effects of block and period × block. No treatment effects were found for DMI, milk yield (41.2 vs. 41.3 ± 2.0 kg/d for CON vs. STAT), milk fat yield, or SCS during the prechallenge period (all $P > 0.10$). There was a treatment × period interaction for protein yield, with STAT decreasing protein yield in period 1 but increasing it in period 2 ($P < 0.01$). Following LPS challenges, challenge quarter SCS response was affected by treatment; in period 1, STAT decreased SCS at 12 h, but in period 2, STAT tended to increase SCS at 12 h (3-way interaction $P = 0.04$). Vaginal temperature was similar across treatments. Whole-udder milk yield was reduced by 7% in the 4 d postchallenge and was not affected by treatment. Despite apparent modulation of immune cell recruitment to the mammary gland following LPS challenge, STAT did not significantly improve whole-body responses to induced mastitis in this model.

Key Words: mastitis, nutritional immunology, direct-fed microbial

1385 Effects of mannanase-hydrolyzed copra meal on production, metabolism, and inflammation in immune-activated lactating dairy cows. L. C. Bombeck^{*1}, E. J. Mayorga¹, B. M. Buol¹, G. J. Combs¹, M. Araba², and L. H. Baumgard¹, ¹*Iowa State University, Ames, IA*, ²*Quality Technology International, Elgin, IL*.

Objectives were to evaluate the effects of mannanase-hydrolyzed copra meal (MCM; QTI, Elgin, IL) on production, metabolism, and inflammation following an i.v. LPS challenge. Twelve Holstein cows (parity 2.5 ± 1 ; 167 ± 3 DIM) were randomly assigned to receive either control (CON; $n = 6$) or MCM (30 g/cow; $n = 6$). Cows were moved to individual box stalls and acclimated for 6 d. The trial consisted of 2 experimental periods (P) during which cows received their respective dietary treatments daily. During P1 (4 d), baseline data were obtained. During P2 (5 d), all cows were challenged with an i.v. LPS bolus (0.08 µg/kg BW). Data were analyzed using PROC MIXED of SAS. The febrile response from LPS peaked at 3 h after bolus (1.4°C , $P < 0.01$) but was unaffected by treatment. The LPS reduced DMI and milk yield (MY) similarly in all cows on d 1 of P2 (38%, $P < 0.01$). During P2, gross feed efficiency (FE) tended to be increased in MCM relative to CON ($P = 0.06$), and ECM FE for MCM was increased relative to CON ($P = 0.04$). Cows in both treatments were hyperglycemic 1 h after LPS (12.6%, $P < 0.01$) and hyperinsulinemic at 2 h (3-fold, $P < 0.01$), which continued through 24 h. Glucagon increased post-LPS and peaked at 2 h in both treatments (57.4%, $P < 0.01$). The LPS altered circulating BUN, BHB, and NEFA, but responses were unaffected by MCM ($P \geq 0.18$). Leukopenia occurred following LPS (83%, $P < 0.01$) similarly between treatments ($P > 0.69$). Circulating LPS-binding protein increased following LPS administration, and this response tended to be attenuated (25%) in MCM compared with CON cows ($P = 0.06$). Relative to P1, serum amyloid A (SAA) and haptoglobin (Hp) concentrations increased similarly in both treatments following LPS (5- and 11-fold at 24 and 72 h, respectively, $P < 0.01$). After the peak, SAA and Hp tended to decrease and returned to baseline quicker in MCM-fed cows compared with CON ($P = 0.11$). In summary, MCM appeared to mitigate the inflammatory response and improve feed efficiency following immune activation.

Key Words: LPS, mannanase-hydrolyzed copra meal (MCM), β -(1→4)-mannobiose

Teaching Undergraduate and Graduate Education: Early Career Dossiers—Documenting Teaching Effectiveness

1386 The importance of teaching experience for academic careers. M. M. Schutz*, *University of Minnesota, St. Paul, MN.*

Documentation of evidence of scholarship in teaching and learning is required for advancing through promotion and tenure in academia. The level of achievement and ways to document scholarship that meets or exceeds expectations differs widely across universities. A sample of guidance documents for evaluation of scholarship in teaching, learning, or instruction was collected from 9 departments of animal science. All documents agreed that delivery of resident credit courses, advising of undergraduate and graduate students, satisfactory student evaluation of instruction, advising, and contributions to curriculum development constituted scholarly activities in teaching. Some institutions included other activities, for example, novel instructional methods, advising undergraduate student clubs, advising undergraduate research, coaching competition teams, and securing external funding of educational programs as scholarly activities. Most documents also required evidence of peer review of teaching, measurement of learning outcomes, and dissemination of pedagogical contributions through publications in scientific or educational journals to measure scholarship of teaching and learning. Nearly all tenure-track positions in animal science programs include a majority or large minority appointment in research as well. Recruiting candidates with substantial evidence of scholarship in teaching and learning is rare, but candidates who have shown interest and gained some experience in scholarly activities related to teaching often predict their future success in teaching. Although experience in teaching is advantageous, interest, passion, innovation, organization, and clarity in presentation are attributes that can also predict achievement in scholarship of teaching and learning.

Key Words: scholarship of teaching and learning, experience, academic career

1387 From barns to brains: How classroom research can be used to improve teaching as early career faculty. Q. K. Kolar*¹ and A. A. Logan², ¹*Cornell University, Ithaca, NY*, ²*Middle Tennessee State University, Murfreesboro, TN.*

Animal science classes and departments around the United States are filled with students who are interested in animals and animal-related careers. Today, students entering animal science programs come to the classroom with a wide range of experiences with large animals. This is likely due to more limited opportunities to interact with large animal species such as horses and dairy cattle reflecting the shift in population to more urban and suburban settings. The introductory animal management courses pose a particular challenge to instructors as they teach students from a variety of backgrounds, majors, experiences, and interests. The objective of this analysis was to survey students enrolled in introductory species-specific animal science courses at Cornell University and Middle Tennessee State University (MTSU) to assess how background experiences in youth programming related to the course content correlate to performance. Students from MTSU Intro to Horse Care and Use (n = 19) and Cornell Dairy Cattle Principles (n = 56) were surveyed at the beginning and end of the Fall 2024 semester to evaluate how their perceptions and experiences changed through the semester. Of the students who completed the survey at the end of the courses, 38.6% (n = 29) participated in one or more youth programs. Of the youth programs

students enrolled in the introductory courses primarily participated in 4-H (n = 23) and FFA (n = 13), and dairy breed or discipline associations (n = 9). Of students who responded they were extremely likely to work in the dairy or equine industry following graduation (n = 23), 78.3% of those students (n = 18) had previously participated in youth programs. Information collected as part of this research is intended to be used to help build collaboration with youth programming and continue to improve course delivery. This work is part of a 3-yr study, with plans for further data to correlate youth involvement with grade success across multiple semesters.

Key Words: education, undergraduate, youth programming

1388 Undergraduate students' demographics and extracurricular involvement are associated with course grades in an introductory food-animal science course. M. Tipton*, J. Ahola, and C. Cramer, *Department of Animal Sciences, Colorado State University, Fort Collins, CO.*

The impact of demographic factors and involvement in extracurricular activities (ECA) on student success are well documented in many science disciplines, yet little data exists for animal science programs. The objective of this study was to determine if demographic factors and ECA involvement were associated with students' final grade in a 100-level Introduction to food animal science course. Students (n = 556) enrolled in the course at Colorado State University during the fall 2021, 2022, and 2023 semesters were anonymously surveyed at semester's end regarding their final grade and ECA participation. Survey items included multiple choice, Likert-scale, and short answer questions. Multivariable logistic regressions assessed associations between final course grade (pass: A, B, or C; fail: D or F; outcome) and high school GPA (HSGPA; ≥ 4.00 , 3.00–3.99, <3.00), ethnicity (0 = non-white; 1 = white), first-generation (FG) status (0 = no; 1 = yes), volunteering to care for university dairy calves (0 = no; 1 = yes), and involvement with undergraduate research (0 = no; 1 = yes). For each association, odds ratios (OR) and 95% CI were reported with the associated P-values. An interaction occurred between FG and HSGPA ($P = 0.03$), which showed that the difference in odds of passing between FG and non-FG students is larger at lower GPA levels. Non-white identifying students had lower odds of passing compared with white identifying students (OR: 0.24, CI: 0.14, 0.39; $P < 0.0001$), and students retaking the course had lower odds of passing the course than first-time enrollees (OR: 0.20, CI: 0.07, 0.59; $P = 0.003$). Students had greater odds of obtaining a passing course grade when they had volunteered to care for university dairy calves (OR: 2.3, CI: 1.4, 3.8; $P = 0.002$) or participated in department research (OR: 8.4, CI: 1.1, 62.0; $P = 0.013$), compared with students who did not. This study highlights the roles of involvement and demographics in academic performance and can be used to inform future student support programs in animal science disciplines.

Key Words: student support, student success, higher education

1389 The role of mentoring in graduate education and career development. T. Martins¹ and A. Faciola*², ¹*The Graduate School, University of Florida, Gainesville, FL*, ²*Department of Animal Sciences, University of Florida, Gainesville, FL.*

Graduate students play a pivotal role in advancing science, education, and extension, particularly in the field of animal and dairy sciences. As expectations on faculty productivity rise, effective mentoring becomes crucial for shaping future professionals and supporting institutional goals. This presentation will emphasize the distinction between academic advisors and mentors, arguing that while advisors guide short-term academic objectives, mentors foster long-term personal and career development. It highlights the importance of providing both roles within graduate programs and training faculty to be effective mentors. Structured mentorship programs, supported by tools like Individual Development Plans and mentor-mentee compacts, can improve student satisfaction and reduce attrition rates. The presentation will highlight how the growing transition of PhD holders from academia to industry

underscores the need for graduate programs to integrate transferable skills into the curriculum. Additionally, intercultural competence and technological proficiency, particularly in artificial intelligence, are identified as essential for students' success in a globalized job market. These efforts also contribute to the long-term success of academic institutions by modernizing graduate programs to prioritize flexible, student-centered mentoring, which prepares highly skilled and highly sought-after graduates who drive innovation and advance research and development in their fields, while also keeping these programs relevant and attractive to employers in a rapidly changing career landscape.

Key Words: career pathways, intercultural competence, transferable skills

Animal Behavior and Well-Being 2

1390 It's all about the cow: US public perceptions toward precision dairy technology use. B. Kelly^{*1}, K. Proudfoot², T. Da Silva³, J. H. C. Costa³, and B. Ventura⁴, ¹University of Tennessee, Knoxville, TN, ²University of Prince Edward Island, Charlottetown, PE, Canada, ³University of Vermont, Burlington, VT, ⁴University of Lincoln, Brayford Pool, Lincoln, UK.

A clearer understanding of public perceptions toward precision livestock farming is needed to ensure that these technologies are socially sustainable. This study sought to (1) characterize public perceptions toward Precision Dairy Technologies (PDT), and (2) describe how perceptions may be influenced by information framing about the potential effects of PDT use. Using a mixed-method online survey distributed to US residents ($n = 2,365$), participants randomly received 1 of 8 vignettes, each containing either positively—or negatively—framed information about hypothetical effects of PDT on human-animal relationships, data transparency, and animal welfare. Here, we focus on presenting the qualitative findings, in which $n = 80$ randomly selected open-ended responses were processed through representational thematic text analysis. From the qualitative data, 6 key themes were identified (1) how PDT affected cows, such as hopes that PDT would positively affect cows or concerns that they would be negatively affected (56% of participant responses addressed this theme); (2) references to technology or data, including optimism about technology use and hopes that data would promote animal monitoring (34%); (3) contingency statements, such as cautious acceptance of PDT as long as the technology did not harm cattle (28%); (4) references to low knowledge, including the desire for more information or acknowledging that their responses were based on assumptions (25%); (5) effects on farmers, such as beliefs that PDT will support farm management farms and better animal care (9%); and (6) consumer implications, such as thoughts on how PDT may impact dairy products and consumption (1%). These results suggest that the dairy industry will benefit from more closely engaging with public concerns regarding potential effects of PDT on animal welfare and the relationship between farmers and animals.

Key Words: social sustainability, precision technology, animal welfare

1391 Computer vision-tracked behavior in dairy calves during a subtropical summer. C. Saha^{*1}, C. G. Savegnago¹, Y. B. Amancio¹, R. M. da Costa¹, J. Gao¹, A. M. Roper¹, T. N. Marins¹, N. Andriotti¹, M. C. Guenther², A. Amato³, J. Laporta², S. Tao¹, and A. A. C. Alves^{1,4}, ¹University of Georgia, Athens, GA, ²University of Wisconsin, Madison, WI, ³Università degli Studi di Messina, Messina, Sicily, Italy, ⁴Institute for Integrated Precision Agriculture, Athens, GA.

This study aimed to investigate the impact of environmental conditions on dairy calves' behavior based on a computer vision system (CVS). The data were collected during 6 d of early July on a commercial dairy farm in southern Georgia. The images were captured every 10 s using 13 bullet cameras (4 MP). Each camera covered 3 hutches, totaling 39 Holstein dairy heifers (14 ± 3.5 d of age). Climate sensors (HOBO) were installed inside some hutches to record microenvironmental conditions. The YOLOV7 algorithm was trained to detect individual calves' behaviors including standing, lying, eating in bucket, and drinking in bucket. We manually annotated 2,000 images randomly selected before the 6-d collection period. This dataset was split into 90% for training and 10% for validation to assess the algorithm performance. The YOLOV7 achieved an average of 92% precision, 89.60% recall,

and 92.20% mean average precision (mAP) for validation images considering all behaviors. Based on these performances, we inferred 6 d of images for computing each behavior pattern every hour, generating 5,041 observations for each behavior class. This dataset was used to estimate the linear effect of the temperature-humidity index (THI) on each behavior trait using a Bayesian mixed model analysis. The 95% highest probability distribution (95% HPD) was adopted to investigate the evidence of a real effect of THI on the studied traits. The mean effect of THI was -1.06 , showing a significant decrease (95% HPD: -1.20 to -0.60) in standing time, while the average effect of THI on lying time was 0.92 min (95% HPD: ~ 0.70 to 1.10), indicating that animals tend to lie down more as THI increases. Besides, the THI effect on eating time (0.05 , 95% HPD: ~ 0.01 to 0.08) and drinking time (0.03 , 95% HPD: ~ 0.01 to 0.04) is positive but small. A weak but significant positive correlation was observed between dew point, drinking, and lying time (ranging from 0.08 to 0.15). Our results show that CVS is a valuable tool for automatic, noninvasive calf monitoring, supporting the need for efficient heat-stress abatement strategies in summer.

Key Words: activity monitoring, artificial intelligence, heat stress

1392 Using a neural network-based approach to identify dairy calf vocalizations and investigate their relationship with temperature during a subtropical summer. B. B. de Medeiros^{*1}, C. G. Savegnago¹, M. C. Guenther², Y. B. Amancio¹, A. M. Roper¹, T. N. Marins¹, N. Andriotti¹, R. M. da Costa¹, J. Gao¹, C. Saha¹, T. Abdulrahman¹, J. Laporta², S. Tao¹, and A. A. C. Alves^{1,3}, ¹Department of Animal and Dairy Science, University of Georgia, Athens, GA, ²Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI, ³Institute for Integrated Precision Agriculture, Athens, GA.

This study proposes an artificial neural network (ANN)-based method to extract and analyze dairy calf vocalizations regarding ambient temperature during a subtropical summer. Data were collected on a dairy farm in South Georgia using portable recorders on 16 hutch-housed Holstein calves. The collection spanned 2 d, totaling 544 h of audio. Hourly temperatures were recorded with a HOBO sensor outside the hutches. A subset of the audio was split into 900 vocalization and 900 background (including neighbor calves' vocalizations) 2-s files for training. Features, including 13 mel-frequency cepstral coefficients, temporal entropy, and pitch, were used for model training. The optimal ANN architecture was selected by cross-validation with grid search. The trained model was used to mine 371,118 2-s fragments (103 h) from 3 calves, which were pruned and merged based on timestamps. Pearson correlation and linear regression analyses (using day period as a covariable) were used to investigate associations with temperature. During cross-validation, the ANN achieved accuracy of 98.61%, precision of 99.43%, recall of 97.75%. On the unlabeled 103-h subset, 703 vocalizations were identified with 34.6% precision. Significant correlations with temperature included duration (-0.307 , $P = 0.0028$), range (-0.468 , $P = 2.54e-06$), kurtosis (-0.338 , P -value 0.0009), zero-crossing rate (-0.305 , $P = 0.003$), maximum amplitude (-0.508 , $P = 2.252e-07$), intensity (-0.385 , $P = 0.0001$), and pitch (0.345 , $P = 0.0007$). Significant negative slopes for duration, range, zero-crossing rate, amplitude, and intensity were found with temperature, while pitch had a positive slope. This method enables vocalization retrieval from large audio datasets under farm conditions, although manual pruning remains necessary. As temperature increased, vocalizations became shorter, lower in intensity, smoother, and higher pitched, possibly reflecting heat stress in calves.

This approach holds promise for future research and large-scale applications with further refinement.

Key Words: activity, audio processing, heat stress

1393 Developing an understanding of the decision-making process of Ontario dairy farmers when managing down dairy cattle: A qualitative study. J. E. Brindle*, M. W. Brunt, D. L. Renuad, D. B. Haley, T. F. Duffield, and C. B. Winder, *University of Guelph, Guelph, ON, Canada*.

The objective of this study was to investigate Ontario dairy farmers' attitudes and perceptions toward down (non-ambulatory) dairy cows, alongside the barriers and facilitators influencing the adoption of best management practices. Four focus groups were conducted in southwestern Ontario in 2024, with 21 total dairy producers participated. Producers were asked about current practices, thoughts on best management practices, challenges to adopting new practices, and about facilities and equipment for down cow care. The focus groups were audio recorded, transcribed, and evaluated qualitatively using applied thematic analysis in Quirkos (version 2.5.3, Quirkos Ltd., 2023) to gather insights into dairy farmers' attitudes, experiences, and decision-making processes. Five core themes emerged: integrated management strategies; farm resources and facilities; veterinary relationships and communication; producer experience and emotional impact; and pain, welfare, and euthanasia considerations. Producers emphasized a balance between proactive, long-term strategies, such as prevention, record-keeping, and research-driven improvements, and the need for flexible, real-time decision making to address unpredictable challenges. Facility design, labor and financial constraints, and veterinary collaboration played important roles in down cow decision making. Further, producers highlighted the emotional burden of making euthanasia decisions, and discussed the need to balance both welfare and economic sustainability. Producers also expressed a need for more research, better diagnostic tools, and practical on-farm solutions to improve down cow management. The interplay of these factors underscores the need for a holistic approach to support producers in navigating the multifaceted challenges and farm specific challenges they face in their daily work. This study contributes to understanding the multidimensional factors influencing dairy producers' management practices for down dairy cows.

Key Words: management practices, recumbent, qualitative

1394 Evaluating welfare in east Tennessee automatic milking herds. J. Ozburn* and E. Eckelkamp, *University of Tennessee, Knoxville, TN*.

Animal welfare has been a focal point of consumer perceptions of dairy farmers across the United States. Animal welfare metrics are subjective and inter- and intra-observer agreement can be challenging. Automatic milking systems (AMS) capture additional data points on individual animals and herd performance and may support welfare evaluations. Our study included 3 AMS dairies in southeast TN ($n = 640$ Holstein cattle) and evaluated lower leg hygiene (LLH), udder hygiene (UDH), upper leg and flank hygiene (ULFH), hock scores, knee scores, locomotion scoring (LS), and BCS based on FARM standards (nationaldairyfarm.com). Farm 1 (F1) had 8 AMS and 480 cows, farm 2 (F2) had 2 AMS and 120 cows, and farm 3 (F3) had 2 AMS with 40 cows. A subset of animals was evaluated every other week from June to December 2024. Herd health, milk quality, visits per day, milk quantity, feed visits, neck activity, and rumination activity were collected from herd and AMS

management systems. To determine base level welfare performance, data were analyzed with the frequency procedure of SAS 9.4 with a Chisq analysis and significance of $P < 0.05$ by farm and by date. A MIXED procedure of SAS 9.4 with significance of $P < 0.05$ was used to further evaluate farm and date impacts on all variables. Overall, ULFH scores were within acceptable welfare limits ($98.9\% \pm 1.5\%$ of herd with score < 2) across all farms. Farm 1 was the only herd outside of acceptable LLH and UDH (52.1% and 17.7%, respectively, scoring ≥ 2). Conversely, hock scores were > 2 on $24.8\% \pm 4.5\%$ of the herds with the F1 having the least hock scores > 2 (18.4%). Knee scores were > 2 in 8.8% of F3. Similarly, F3 had the poorest LS with 54.9% of the herd scoring ≥ 2 . Across all herds, BCS never dropped below 2 on a scale of 1 to 5. Overall, F1 had significantly higher hygiene (1.94 ± 0.01) than F2 and F3 (1.22 ± 0.01 and 1.25 ± 0.02 , respectively). Farm 3 had significantly higher LS and knee scores (2.40 ± 0.03 and 1.60 ± 0.02 , respectively) compared with F1 (1.09 ± 0.02 and 1.13 ± 0.01 , respectively) and F2 (1.06 ± 0.02 and 1.25 ± 0.02 , respectively). Over time, the mean scores per date remained similar. Future analyses will determine potential of AMS captured variables to detect differences in welfare states across herds.

Key Words: hygiene, locomotion, body condition score

1395 Calf welfare and husbandry practices on dairy-beef operations in the Midwestern United States. E. M. Schafer*, K. C. Creutzinger², M. M. Woodrum Setser², G. G. Habing³, M. A. Erasmus¹, K. D. Vogel⁴, and J. A. Pempek⁵, ¹Purdue University, West Lafayette, IN, ²University of Vermont, Burlington, VT, ³The Ohio State University, Columbus, OH, ⁴University of Wisconsin–River Falls, River Falls, WI, ⁵USDA-ARS Livestock Behavior Research Unit, West Lafayette, IN.

The US dairy-beef industry lacks health and welfare metrics for calves in the first weeks of life. Therefore, the objectives of this study were to (1) establish prevalence estimates for key health and welfare measures for dairy-beef calves at 1, 5, and 10 wk after arrival to rearing facilities in the Midwestern United States, and (2) describe calf husbandry practices across these operations. From February 2024 to January 2025, 72 calves per farm (~ 3 –7 d of age) from 20 calf-raising facilities ($n = 1,436$ calves) in Indiana, Ohio, Wisconsin, and Iowa were enrolled. Calves were assessed for signs of dehydration, diarrhea, navel inflammation, bovine respiratory disease (BRD), and hygiene at 1 and 5 wk, and signs of BRD and hygiene at 10 wk. Descriptive statistics were used to estimate the prevalence of abnormal measures and describe husbandry practices across facilities. Given clustering within farms, 95% CI for prevalence estimates were calculated using PROC SURVEYFREQ with farm specified as the cluster variable in SAS 9.4. At 1 wk, 67.3% (95% CI: 56.5%–78.1%) of calves had diarrhea, 20.6% (12.7%–28.6%) had BRD, 9.5% (3.9%–15.0%) had navel inflammation, and 8.2% (3.9%–12.5%) had dehydration. At 5 wk, 29.2% (18.9%–39.6%) of calves had BRD, 16.8% (9.7%–23.9%) had diarrhea, and 9.4% (5.8%–13.0%) had poor hygiene. At 10 wk, 25.1% (17.3%–33.0%) of calves had BRD and 15.6% (7.3%–23.9%) had poor hygiene. Farm-level disease prevalence varied widely across facilities; diarrhea prevalence at 1 wk ranged from 14.5 to 100% and BRD at 5 wk from 1.4 to 76.6%. Thirty percent of farms housed preweaning calves socially (14 ± 2.4 calves/group). Across farms, 35% of calves were dairy $\times$ beef. Nearly one-third of crossbred calves were female, while all purebred dairy calves were male. Average milk allowance was 4.8 ± 0.3 L/d, and mean weaning age was 44 d (range: 38 to 56 d). Approximately half of facilities administered antibiotics via water to all calves on arrival for 9 ± 4.5 d. These results demonstrate high prevalences of diarrhea at 1 wk and BRD at 5 and 10

wk in dairy-beef calves, and significant variation among farms in health measures and husbandry practices.

Key Words: calf health, dairy-beef, management

1396 Tracking stress: Changes in salivary cortisol and cortisone levels in preweaning Holstein heifers from thermoneutral to heat stress conditions. A. Johnson*, B. Goncalves da Costa, A. Jannat, A. Schilling-Hazlett, S. Bowman, and J. Vargas, *Colorado State University, Fort Collins, CO*.

Salivary cortisol and cortisone are reliable biomarkers of heat stress in mammals. Young calves may experience heat stress when the temperature-humidity index (THI) exceeds 65. However, the daily dynamics of these biomarkers. This study aims to describe salivary cortisol and cortisone dynamics in preweaning Holstein heifers during time of the day considered thermoneutral ($\text{THI} \leq 65$) or heat stress ($\text{THI} > 65$) conditions. Thirty Holstein female calves, ~14 d old, were housed individually in a single row of hutches at a commercial dairy farm in Colorado. Saliva samples were collected from each calf at time points 0500, 1000, and 1400 h on d 1 (July 17, 2024) and again on d 2 (July 25, 2024). To collect saliva, a 65-mm foam plug was used for the calves to chew for at least 1 min. After sample collection, the plugs were placed in 50-mL conical tubes and centrifuged at $2,000 \times g$ for 5 min. During both collection days, sampling was conducted at least 2 h before feeding, which were scheduled by farm operation at 0700, 1200, and 1800 h. Recovered saliva was stored at -20°C and cortisol and cortisone were quantified using UPLC-MS/MS. A weather station (U30, HOBO data loggers, MA) located next to the row of hutches monitored THI. Mixed models were used to determine the least squares means of cortisol and cortisone, considering time point and maximum THI of the day before sampling. The maximum THI the day before of d 1 was 76.8, whereas for d 2 it was 79.2. Calf ID was considered as a repeated measure, whereas maximum THI was a random effect. Both 0500 h sampling points were considered thermoneutral (avg: 61.8; std: 3.89 units), while both 1000 and 1400 h sampling points were considered heat stress (74.06; 0.41 and 77.56; 1.25 units). We observed significantly higher cortisol levels at 0500 h compared with 1000 h (0.3 ± 0.02 vs. 0.18 ± 0.02 ng/mL), whereas no differences were found between 0500 and 1400 h (0.23 ± 0.02 ng/mL), and between 1000 and 1400 h. Regarding cortisone levels, we observed the same associations with 0500 h (0.64 ± 0.05 ng/mL) having greater saliva concentrations compared with 1000 h (0.42 ± 0.06 ng/mL), and no differences between 0500 and 1400 h (0.61 ± 0.05 ng/mL), and between 1000 and 1400 h. The main effect of maximum THI the day before sampling was associated with cortisol and cortisone concentrations. Thus, samples from d 2 had greater overall cortisol (0.27 ± 0.02 vs. 0.2 ± 0.02 ng/mL) and cortisone (0.65 ± 0.04 vs. 0.47 ± 0.05 ng/mL) concentrations compared with samples from d 1. Salivary cortisol and cortisone concentrations showed a circadian pattern with higher levels during thermoneutral sampling times. These results might be associated with lagged adrenal cortex responses to the levels of thermal stress on the previous day, as higher overall cortisol and cortisone were observed in d 2, which had greater maximum THI. Additionally, cortisone appears to be a useful biomarker for heat stress in preweaning Holstein heifers.

Key Words: thermal stress, thermal biomarkers, heifer calves

1397 Plasma proteomic shifts from thermoneutral to heat stress conditions in preweaning Holstein heifers. D. Manriquez*, A. Johnson, A. Jannat, M. Swenson, A. Schilling-Hazlett, and S. Bowman, *Colorado State University, Fort Collins, CO*.

Advanced proteomics techniques enable detailed characterization of multiple physiological mechanisms involved in heat stress response. However, plasma proteomic profiles in dairy heifer calves have been scarcely explored. This study aimed to assess differences in the plasma proteome of preweaning Holstein heifers transitioning from thermoneutral to heat stress conditions within the same day. Ten preweaning Holstein heifers from a commercial dairy farm in northern Colorado were enrolled in a prospective crossover study. Blood was collected at 7 d of age at 0500 h (thermoneutral, $\text{THI} = 59$) and 1400 h (heat stress, $\text{THI} = 80$) from the jugular vein using 9-mL EDTA tubes. Plasma was harvested and stored at -80°C until analysis. Samples were processed and analyzed at the Analytical Resource Core at Colorado State University using the Enrich-IST kit (PreOmics, Germany) for sample preparation and mass spectrometry analysis (LC-MS/MS) and Data Independent Acquisition methods for protein identification. The *Bos taurus* proteome was used as reference with a false discovery rate set at 0.01. Differential expression analysis was conducted, with statistical significance defined by a corrected P -value ≤ 0.05 and a Log2 fold change of ≤ -1 or ≥ 1 . A total of 1,304 proteins were identified, with 52 showing significantly different abundance between thermoneutral and heat stress conditions. Of the 52 proteins, 46 were greater in the thermoneutral samples and 6 were greater in the heat stress samples. Overall, proteins related to nuclear cell integrity (H2AC20; H1-3), glucose metabolism (BPGM), and intracellular proteolysis (PSMC1) were differentially expressed during heat stress conditions, while those associated with synaptic vesicle transmission (VAMP2), coagulation (RASGRP2), and neuroprotection (RTN3) were greatly expressed under thermoneutral conditions. Plasma proteomic analysis offers opportunities to identify specific cellular damage and functional pathways, as well as to understand the transient and long-term effects of heat stress. This can potentially inform genetic selection strategies and supplementation tools for preweaning dairy heifers exposed to elevated thermal conditions.

Key Words: proteome, heat stress, heifers

1398 Effects of heat stress on physiological indicators and weight gain in preweaning dairy heifer calves in a humid continental climate. L. M. Conrad*, D. C. Tulpan¹, V. Ouellet², and T. J. DeVries¹, ¹Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, ²Department of Animal Sciences, Université Laval, Laval, QC, Canada.

Calves raised in humid continental climates experience intermittent exposure to high temperatures and humidity for several months a year. Our objective was to determine whether this can trigger the consequences associated with chronic heat stress. Holstein heifer calves ($n = 138$) were enrolled at birth from 2 Ontario, Canada, commercial dairy farms between May and August 2024. Farms were visited 3×/wk and calves weaned at ~10 wk of age. Calves were weighed weekly to calculate ADG. Calf rectal temperature and respiratory rate were measured at each visit and averaged per week. Data loggers, placed within pen at calf level, recorded ambient temperature and relative humidity at 10-min intervals. Temperature-humidity indices (THI) were calculated, including the average weekly THI, weekly maximum THI, and the average hours per day per week for which THI exceeded the thresholds of 65 and 69. Data were analyzed using repeated measures, mixed-effect linear regression models, with farm and calf within farm as random effects. Weekly THI averaged 63.7 ± 5.7 (mean $\pm$ SD), ranging from 50.1 to 75.2. Weekly maximum THI averaged 76.8 ± 4.4 , ranging from 66.6 to 88.3. The average time per day (and range) above the THI thresholds of 65 and 69, were 12.8 ± 5.7 h/d (0.5–23.2 h/d) and 7.4 ± 4.8 h/d (0–20.4 h/d), respectively. Calf rectal temperature, respiratory rate, and ADG averaged

39.2 ± 0.4°C, 54 ± 12.4 breaths/min, and 0.75 ± 0.56 kg/d, respectively. Each 1 h increase in the time (h/d) THI exceeded 69, rectal temperature (±SE) increased by 0.01 ± 0.004°C ($P = 0.02$). Each 10-unit increase in the weekly average and maximum THI, respiratory rate increased by 5.4 ± 1.5 breaths/min ($P < 0.01$) and 14.1 ± 1.9 breaths/min ($P < 0.01$), respectively. Each 1 h increase in time (h/d) THI exceeded 65 and 69, respiratory rate increased by 0.7 ± 0.1 breaths/min ($P < 0.01$) and 1.0 ± 0.1 breaths/min ($P < 0.01$), respectively. Each 10-unit increase in the weekly average THI and maximum THI, ADG was reduced by 0.13 ± 0.04 kg/d ($P < 0.01$) and 0.21 ± 0.05 kg/d ($P < 0.01$), respectively. Each 1 h increase in the time (h/d) THI exceeded 69, ADG was reduced by 0.01 ± 0.005 kg/d ($P = 0.03$). Overall, intermittent exposure to high temperatures and humidity in a humid continental climate lead to physiological changes and reduced ADG in calves, highlighting the need to manage heat stress to optimize performance.

Key Words: heat stress, weight gain, physiological indicators

1399 Effects of short-term cold exposure on *Bos taurus* dairy calves carrying the SLICK gene. G. M. Worth*, E. G. Donkersloot, O. K. Spaans, R. J. Spelman, and S. R. Davis, *Livestock Improvement Corporation, Hamilton, Waikato, New Zealand*.

The SLICK genetic variant is a dominant mutation in the prolactin receptor (PRLR) on BTA20 associated with a short hair coat and improved ability to lose heat in *Bos taurus* cattle. This raises questions as to the viability of SLICK cattle in temperate climates where temperatures can be relatively extreme in both winter and summer. The objective of this study was to investigate the thermoregulatory responses of SLICK ($n = 12$) and control calves ($n = 12$) during short-term cold challenges in

a modified refrigerated container. Calves were tested with both wet and dry hair coats at 1 wk of age, with testing repeated at 5 wks of age. On each experimental day, after morning milk feeding, calves (2 SLICK and 2 controls) were penned at a temperature of -4.5°C between 0900 and 1500 h. Hair length, rectal temperature, skin surface temperature, and behavioral measurements (shivering, lying, and piloerection) were evaluated hourly. Infrared images (FLIR ONE Pro for iOS) were collected from the eye, nose, rump, and lower leg every 1 h and the maximum temperature (°C) determined using FLIR Thermal Studio, version 1.9.70. Statistical analyses were performed in SAS (version 3.81) using the FREQ procedure for the binary response variables of shivering, piloerection, and lying behavior. Temperature responses were analyzed using the MIXED procedure with repeated measurements. Hair length differed ($P < 0.05$) between SLICK and control calves at all locations: neck (20.2 ± 4.24 mm vs. 27.3 ± 6.22 mm), pin bone (18.2 ± 3.46 mm vs. 25.6 ± 6.75 mm), and shoulder (21.5 ± 4.54 mm vs. 26.2 ± 6.39 mm), respectively. Rectal and skin surface temperatures of SLICK and control calves declined in response to cold. Rectal temperatures decreased by -0.12°C ± 0.22 in SLICK calves and -0.22°C ± 0.20 in controls, while leg surface temperatures dropped by -9.52°C ± 2.37 and -10.68°C ± 2.36, respectively. There were no differences in responses between the 2 genotype groups at either age or with wet and dry treatments. The SLICK calves showed an increased frequency of shivering at both 1 ($P < 0.001$) and 5 ($P < 0.001$) weeks. There was no difference in piloerection or lying behavior between SLICK and control calves. Results indicate that SLICK calves can effectively maintain their body temperature during short-term cold stress and show an increased propensity to shiver.

Key Words: SLICK calves, cold stress

Animal Health 4: Colostrum

1400 Does a supraphysiological oxytocin injection after parturition affect blood-milk-barrier closure and IgG transfer into milk in dairy cows? E. C. Kessler, R. M. Bruckmaier, and J. J. Gross*, *Veterinary Physiology, Vetsuisse Faculty, University of Bern.*

Oxytocin (OT) can affect the blood-milk-barrier integrity by modulating the closure of tight junctions. Our study aimed at retarding the postpartum closure of the blood-milk-barrier and concomitantly prolonging the transfer of IgG into colostrum of dairy cows by a supraphysiological OT injection. Multiparous Holstein cows ($n = 28$) were enrolled. Blood and milk samples were collected at the first 2 milkings after parturition and milk yields were recorded. Ten cows received 50 IU OT i.v. at 30 min after the first milking (OT+30), 9 cows were injected 50 IU OT i.v. at 120 min before the second milking (OT-120), and 9 cows remained untreated (CTRL). Cows were milked at 6.7 ± 0.7 h after parturition (first milking) and 11.5 ± 0.3 h later (second milking). Milk was analyzed for fat, protein, lactose, and IgG, serum albumin (SA), and electrolytes (Na, K, Cl) concentrations and for lactate dehydrogenase (LDH) activity. Concentration of α -LA was measured in blood plasma. Data were evaluated using a mixed model (SAS) with group, milking number, and group $\times$ milking number as fixed effects, and cow as random subject. In all groups, concentrations of protein, IgG, SA, LDH in milk decreased from first to second milking, while lactose content in milk increased ($P < 0.05$). Milk yield and milk fat content did not differ between first and second milking ($P > 0.05$). Concentration of α -LA in plasma tended to decrease from first to second milking. Treatments did not affect changes in blood and milk from first to second milking. In conclusion, a single injection of supraphysiological OT either at 30 min after the first milking p.p. or 2 h before the second milking p.p. did not retard the closure of the blood-milk-barrier in dairy cows. Furthermore, OT application did not prolong the active transport of IgG into milk after the first milking.

Key Words: blood-milk barrier, oxytocin, colostrum

1401 Effects of freezing on the microbiome of bovine colostrum from organic and conventional dairies. M. Miranda^{*1,2}, E. M. Abdelfattah^{2,1}, B. Oakley², J. Peralta², and J. Reynolds², ¹*Cal Poly Pomona*, ²*College of Veterinary Medicine, Western University.*

Maternal colostrum is essential for calf health, development of the immune system, and establishment of the gastrointestinal microbiome. Colostrum has traditionally been frozen to prevent alterations to its composition. However, little research exists regarding the effects of freezing on the taxonomic composition of the colostrum microbiome. Therefore, the objective of this study was to characterize the colostrum microbiome collected from conventional and organic dairies, before and after freezing. Colostrum samples were collected at the first milking after parturition from 15 conventional dairies in the Central Valley of California and 5 organic dairies in northern California, Colorado, and Texas. The herd size of dairies ranged in size from 100 to 20,000 cows. A total of 5 colostrum samples (50 mL each) were collected per 100 cows, with a maximum of 20 samples per dairy. Colostrum quality was measured by Brix refractometer at the time of collection. Each colostrum sample was split into two 10-mL aliquots: one designated as fresh, which was processed immediately, and the other designated as frozen, which was stored at -20°C for a period of 24 to 122 h. Both fresh and frozen colostrum samples were treated for DNA extraction followed by SYBR green qPCR to determine bacterial abundance. High-throughput sequencing of 16S rRNA gene amplicons was also performed using the Illumina

MiSeq v2 600 cycle kit to characterize the microbial community. In addition, aerobic plate counts were conducted using 3M Petrifilm to compare bacterial concentrations in colostrum. Pairwise t -tests showed that colostrum from organic dairies had significantly higher ($P < 0.01$) brix values than colostrum from conventional dairies. The community composition of the colostrum microbiome also varied significantly by dairy and dairy type (organic vs conventional; PERMANOVA test, $P < 0.01$). Interestingly, freezing did not significantly affect the composition of the colostrum microbiome, or the number of viable cells as measured by qPCR or aerobic plate counts. These findings suggest that freezing is a viable method for colostrum storage without altering its microbial composition or viability.

Key Words: colostrum, freezing, microbiome

1402 Effects of feeding colostrum volumes proportional to birth body weight on efficiency of IgG absorption and gastric emptying in Holstein calves. G. Frederick^{*1}, M. Wieland¹, A. Singh¹, R. Ewing¹, H. Somula¹, M. A. Steele², and S. Mann¹, ¹*Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY*, ²*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

Our objective was to evaluate the effects of feeding colostrum at different volumes on IgG absorption and gastric emptying. Female Holstein calves ($n = 88$) were enrolled in a randomized block design to receive colostrum volumes corresponding to 6%, 8%, 10%, or 12% of their birth body weight (BBW). A subset of calves ($n = 32$) received colostrum with a gastric emptying marker acetaminophen (ACE) at 150 mg/kg metabolic BW (BBW^{0.75}). Colostrum was administered ≤ 2 h of birth via esophageal tube feeder. Blood samples were collected before feeding (0 h) and at 24 h. For the ACE dosed calves, plasma samples were also collected at 2, 4, 6, 8, 10, and 48 h and ACE concentrations were determined via a colorimetric assay. Colostrum and 24 h serum IgG concentrations were measured using RID, and apparent efficiency of absorption (AEA) of IgG was calculated at 24 h. A mixed-effects ANOVA included treatment as a fixed effect, block as a random effect, and BBW as a covariate. Results are reported as LSM with 95% CI. The mean (range) BBW (kg) was 40.0 (31.8–49.1). Pooled colostrum had a median (range) Brix value (%) of 24.2 (21.2–29.6) and IgG concentration (g/L) of 86.7 (73.2–121.1). Calves in the 6%, 8%, 10%, and 12% groups received median (range) colostrum volumes (L) of 2.3 (2.0–2.9), 3.1 (2.5–3.9), 4.0 (3.5–4.5), and 4.8 (4.2–5.6). Serum IgG (g/L) at 24 h was lowest in the 6% group (28.8 [25.8–31.8]), rising to 37.4 (34.4–40.4) in the 8% group, and peaking in the 10% and 12% groups (41.1 [38.1–44.1] and 43.4 [40.4–46.4], respectively). Apparent efficiency of absorption (%) declined with feeding rate; it was highest in the 6% and 8% groups (47.8 [44.9–50.7]; 46.2 [43.3–49.0]), decreasing to 41.0 (38.1–43.9) in the 10% group, and lowest in the 12% group at 36.3 (33.5–39.2). Plasma [ACE] (mg/L) was higher in the 6% group (25.3 [22.6–28.0]) than in the 12% group (20.9 [18.1–23.6]), with no differences among the other groups. Feeding a single colostrum meal at 8% to 10% of BBW within 2 h after birth optimizes IgG absorption. Feeding at 12% BBW provides only a slight increase in [IgG] and reduces AEA and gastric emptying.

Key Words: colostrum, volume, birth body weight

1403 Impact of butyrate and monensin supplementation in colostrum on passive immunity transfer. G. Keshavarz^{1,2}, K. R.

Johnston^{1,2}, J. van Niekerk^{2,1}, and A. H. Laarman^{*1,2}, ¹University of Alberta, Edmonton, AB, Canada, ²Lakeland College, Vermillion, AB, Canada.

Immunoglobulin-G absorption in neonatal calves is limited to the first 24 h, likely due to intestinal maturation. Gut closure is accelerated with butyrate-supplemented colostrum, possibly due to butyrate's role in cell maturation. This study investigated the effects of supplemental butyrate or monensin in colostrum replacer (CR) on the efficacy of IgG absorption in newborn calves. Newborn bull calves (n = 36) were assigned to either a baseline group or one of 3 treatment groups. Baseline calves were not fed CR and were slaughtered at 1 h of life (BASE). Calves in the treatment groups received 1.3 L of CR (200 g IgG) with 150 mg/kg BW acetaminophen and either no supplement (CON), 2.5% wt/vol Na-butyrate (BUT), or 1 mg/kg BW monensin (MON) at 1 h of life, and were slaughtered at 12 h. Colostrum osmolality was adjusted to 400 mOsm/kg using saline. Blood samples were taken every 30 min from 2 to 8 h and hourly from 8 to 12 h. Blood and tissues were collected at harvest for analysis. Statistical analyses in RStudio used one-way and 2-way ANOVA. No differences were observed among the MON, CON, and BUT groups for serum IgG concentration ($P = 0.60$). Villi length, crypt depth, and villi to crypt (VtoC) ratio throughout the entire small intestine were assessed to study intestinal histomorphology. Calves from the BASE group had lower VtoC ($P < 0.0001$) compared with all treatments. The villi height differed by treatment ($P < 0.001$), where MON had shorter villi than CON ($P = 0.001$). Crypt depth for BUT was higher than MON ($P < 0.05$) only in the proximal and distal jejunum. The BASE group had the highest liver glycogen concentrations ($P < 0.01$), while no differences were observed among the other treatment groups. MON group had the highest maximum plasma acetaminophen concentration ($5.1 \pm 1.2 \mu\text{g/mL}$), and longest time to peak concentration ($562.5 \pm 47 \text{ min}$), along with greater cumulative acetaminophen recovery ($P = 0.02$). No differences were observed among the MON, CON, and BUT groups in abomasal emptying rate. These results suggest that although serum IgG levels remained unchanged, MON treatment showed signs of delayed intestinal maturation.

Key Words: butyrate, monensin, colostrum supplementation

1404 Randomized clinical trial comparing doses of colostrum IgG to maximize transfer of passive immunity in calves. D. A. Hapukotuwa^{*1}, J. House¹, K. Denholm², and S. Rowe¹, ¹Sydney School of Veterinary Science, The University of Sydney, Camden, NSW, Australia, ²School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, Glasgow, Scotland.

The optimal colostrum IgG dose to feed calves, particularly when supply is limited, remains unclear. Our aim was to investigate the biologically optimal dose (i.e., the mass of oral IgG which maximizes serum IgG) and the maximum practically feasible (MPF) dose of IgG to feed newborn calves. A randomized clinical trial was conducted in a herd where cows were milked within 2 h of calving; colostrum was pooled, pasteurized in 40-L batches, and calves were fed up to 4 L of colostrum. Calves were block-randomized to receive an oral colostrum IgG dose of 200 (n = 45), 250 (n = 125), 300 (n = 123), or 350g (n = 107) in a single feeding within 2 h of birth. Doses were titrated after measuring colostrum IgG concentration using radial immunodiffusion (RID). Calf serum was collected at 48 h and tested for IgG concentration using RID. The IgG concentrations and volumes of all colostrum harvested were recorded and used to estimate the MPF dose per calf. Linear models and estimated marginal means (EMM) were used to compare serum IgG among treatment groups. Serum IgG increased linearly with higher oral IgG doses. The EMM IgG (g/L) for each group were 24.8 (95% CI: 21.9, 27.8),

29.6 (95% CI: 23.4, 35.8), 32.5 (95% CI: 26.3, 38.8) and 36.5 (95% CI: 30.2, 42.7) for 200, 250, 300, and 350 g doses respectively, suggesting the biologically optimal dose is >350g. The MPF dose was 320 g per calf when feeding up to 4 L of colostrum. This was based on the finding that the mean IgG available per calf during the study was 320 g (118 kg IgG shared among 368 calves), and that most colostrum batches (89%) would be able to deliver 320 g when limited by batch concentration (range: 282–482 g) and dose ($\leq 4 \text{ L}$). Producers should aim to feed as much IgG as practically possible. Our findings indicate that it is feasible to feed calves a single dose of 320 g under the conditions of this study, involving a single feeding of up to 4 L within 2 h of birth. This number may differ between farms depending on IgG mass harvested per cow.

Key Words: colostrum, IgG, calves

1405 Comparing the correlation between serum total solids and serum IgG in Holstein and Angus crossbred newborn calves. D. A. Hapukotuwa^{*1}, J. House¹, K. Denholm², and S. Rowe¹, ¹Sydney School of Veterinary Science, The University of Sydney, Camden, NSW, Australia, ²School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, Glasgow, Scotland.

Our objective was to identify if the relationship between serum TS and serum IgG differed between Holstein and Angus-Holstein cross (Angus X) calves. This observational study was nested within a clinical trial where calves were randomized to colostrum IgG doses ranging from 200 to 350 g. Calf blood was collected, centrifuged, and tested for serum total solids (STS) using optical refractometry at 48 h of age. The remaining serum was stored frozen at -20°C for further testing via radial immunodiffusion. Multivariable linear models were used to compare serum IgG and STS between breeds. Area under the curve (AUC) and diagnostic sensitivity (Se) and specificity (Sp) were used to measure the ability of STS to identify calves with serum IgG $\geq 25 \text{ g/L}$ (an industry target). The number of calves analyzed in the final dataset from each group were 274 Holstein and 126 Angus X. The mean (SD) serum IgG and STS was 32.1 g/L (8.0) and 61.0 g/L (6.1) for Holstein and 30.2 g/L (7.3) and 57.1 g/L (5.4) for Angus X calves. Although serum IgG was similar between breed, Holstein calves had 3.1 g/L (95% CI: 2.2, 3.9) higher STS than Angus X calves after adjusting for colostrum dose, time to feeding, and volume fed. The AUC of STS for predicting IgG $\geq 25 \text{ g/L}$ in both breeds was 0.86. A cut-point of 62 g/L STS to predict IgG $\geq 25 \text{ g/L}$ in Holsteins had 51% Se and 89% Sp. However, we found that a cut-point of 59 g/L STS was better able to predict IgG $\geq 25 \text{ g/L}$ in Angus X (Se 77%, Sp 75%) compared with 62 g/L STS (Se 38%, Sp 96%). Although colostrum IgG absorption is equivalent between breeds, the correlation between serum IgG and STS differs, likely due to Angus X calves having a lower concentration of non-IgG proteins in serum. We believe a lower cut point of >59 g/L may be necessary to predict a calf serum IgG $\geq 25 \text{ g/L}$ for Angus X calves, compared with the >62 g/L recommendation proposed for Holsteins in the literature.

Key Words: serum IgG, Holstein, Angus-Holstein cross (Angus X)

1406 Colostrum's role in shaping immune function in neonatal dairy calves. M. Cid de la Paz^{*}, F. Viquez-Umana, M. Mancheno, T. Fernandez-Wallace, F. Peñagaricano, H. Mantovani, and L. R. Cangianno, Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.

This study investigates the effect of colostrum on the development of the main T lymphocyte populations during the first month of life. Twenty-four Holstein calves were randomly assigned to one of 3 treatments in

a completely randomized block design: fresh colostrum (FSH, $n = 8$), frozen colostrum (FRZ, $n = 8$), or colostrum-deprived (DPR, $n = 8$). FSH and FRZ calves received colostrum ($>22\%$ Brix) equivalent to 8% of BW within the first 3 h of life and 6% of BW at 8–12 h after birth, while DPR calves were fed milk replacer at the same amount. After d 1 all calves were fed a common milk replacer (26% protein, 20% fat, Land O'Lakes) until d 28. Blood samples were collected from the jugular vein at birth and on d 2, 7, 14, and 28. Flow cytometry was used to assess the proportions of $CD4^+$, $CD8^+$, $\gamma\delta$ T cells, as well as the memory marker CD45RO. Data were analyzed using R Studio 4.4.1 with a linear mixed model. No significant differences were observed between treatments on d 0 and 2. However, DPR calves had significantly lower proportions of $\gamma\delta$ T cells on d 7 and 14 ($49.5\% \pm 3.3\%$ and $46.0\% \pm 3.3\%$, respectively) compared with colostrum fed calves ($57.0\% \pm 3.3\%$ and $53.8\% \pm 3.3\%$; $P < 0.01$), accompanied by a compensatory increase on d 7 and d 14 in $CD4^+$ T cells ($21.0\% \pm 2\%$ and $19.6\% \pm 2\%$ in DPR vs $13.8\% \pm 2\%$ and $14.2\% \pm 2\%$ in colostrum-fed calves) and $CD8^+$ T cells ($12.3\% \pm 1.2\%$ and $13.0\% \pm 1.2\%$ in DPR vs $9.0\% \pm 1.2\%$ and $10.3\% \pm 1.2\%$ in colostrum-fed calves; $P = 0.07$). Additionally, FSH calves exhibited a lower proportion of the memory marker CD45RO in $\gamma\delta$ T cells on d 28 ($29.0\% \pm 3.8\%$) compared with FRZ ($40.2\% \pm 3.8\%$; $P = 0.05$). In neonatal calves, $\gamma\delta$ T cells are the predominant lymphocyte subset in systemic circulation but progressively decline over the first 6 mo of life. These findings indicate that colostrum delivers critical signals that sustain $\gamma\delta$ T cell prominence during early life. Deprivation of colostrum disrupts this balance, leading to a compensatory expansion of $CD4^+$ and $CD8^+$ T cell populations while dampening $\gamma\delta$ T cell activation. Notably, colostrum freezing also appears to influence early T cell function, shaping the development of memory T cells.

Key Words: calf, health, T lymphocytes

1407 The effect of colostrum deprivation on the gut microbial dynamics of beef-on-dairy calves. R. Santos^{*1}, K. Holzapfel¹, S. Gade¹, L. Kluppel¹, D. Morais², C. Rocha², V. Machado², M. Ballou², R. Broadway³, N. C. B. Sanchez³, and F. Rosa¹, ¹*School of Veterinary Medicine, Texas Tech University, Amarillo, TX*, ²*Department of Veterinary Sciences, Texas Tech University, Lubbock, TX*, ³*Livestock Issues Research Unit, ARS, USDA, Lubbock, TX*.

Initial gut microbiota development is crucial for nutrient absorption and protection against infections. Colostrum ingestion plays a crucial role in this process. In addition to promoting immunity, colostrum promotes gut microbiota colonization by facilitating the growth of beneficial species. In this study, we examined the effect of colostrum deprivation on the microbial community in neonatal beef-on-dairy calves exposed to *Salmonella Typhimurium*. Newborn crossbred beef $\times$ dairy calves ($n = 24$) were enrolled in this study until 21 d of age. After birth, calves were randomly assigned to a colostrum-deprived or colostrum-fed group. A subset of calves in each group was orally inoculated with *Salmonella Typhimurium* at 8 d of age. Thus, the experimental groups were colostrum-deprived non-*Salmonella* ($n = 4$), colostrum-deprived *Salmonella* ($n = 4$), colostrum-fed non-*Salmonella* ($n = 6$), and colostrum-fed *Salmonella* ($n = 6$). Meconium samples were collected from all calves within 24 h after birth. At 7, 8, 9, 10, 11, 14, and 21 d of age rectal swabs were collected from all calves and stored at -80°C until metagenomics analyses. The V1-V3 region of 16S rRNA gene sequencing was conducted using Illumina MiSeq platform. Relative abundance and group differences were measured using ANOVA. Statistical significance was declared at $P \leq 0.05$. A reduction in microbial richness (Shannon Diversity) was observed in the colostrum-deprived *Salmonella* calves compared with the colostrum-fed *Salmonella* group

($P = 0.02$). Overall, greater abundance of *Fusobacterium mortiferum* and *Lachnospiraceae* was observed in the colostrum-deprived relative to the colostrum-fed calves ($P < 0.01$) regardless of *Salmonella* inoculation. At the phylum level, *Firmicutes* and *Bacteroidetes* decreased in the colostrum-deprived *Salmonella*-inoculated calves ($P < 0.01$) post-*Salmonella* inoculation. These data suggest that colostrum feeding affects specific microbial taxa that influences gut health. This initial colonization is crucial for the development of a stable and functional gut microbiota in beef-on-dairy calves.

Key Words: beef $\times$ dairy calves, diet, microbiome

1408 Epidemiologic risk factors for the PCR-based identification of *Salmonella* Dublin on dairy-beef farms. S. A. Arevalo¹, J. Kopkey², J. A. Pempek³, S. Locke¹, and G. Habing^{*1}, ¹*Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH*, ²*Four Star Veterinary Clinic, Chickasaw, OH*, ³*Livestock Behavioral Research Unit, USDA-ARS, West Lafayette, IN*.

This study aimed to assess the prevalence and identify risk factors associated with PCR-based detection of *Salmonella* Dublin within environmental samples on dairy-beef farms. We hypothesized that specific farm management practices and environmental conditions would be significantly associated with the presence of *S. Dublin*. A cross-sectional study was conducted on 63 dairy-beef farms across 7 states from June 2023 to November 2024. Environmental boot swabs ($n = 243$) were collected from preweaning alleyways, milk mixing rooms, and post-weaning pens. Samples were analyzed using an in-house multiplex PCR targeting *S. Dublin*-specific gene markers. Additionally, a questionnaire was administered to collect data on farm demographics, management, calf movement, and biosecurity. A directed acyclic graph (DAG) was used to map relationships between potential risk factors and *S. Dublin* presence. Descriptive statistics were performed, followed by logistic regression assuming a binomial distribution. *Salmonella* Dublin was detected in 33.3% (21/63) of dairy-beef farms and 19.8% (48/243) of environmental samples. Most positive farms (56.5%, 13/23) had only one positive sample. The similar prevalence ($\sim 20\%$) observed across sample locations suggests widespread environmental contamination. The final multivariable model identified frequent calf introduction ($P < 0.0007$) and sourcing animals from auctions or calf dealers ($P < 0.001$) as significant risk factors for *S. Dublin* detection. There was not sufficient evidence of an association between pathogen presence and farm size or location. This study demonstrates a considerable prevalence of *S. Dublin* in dairy-beef farm environments. The identified risk factors emphasize the role of calf introduction frequency and sourcing practices in *S. Dublin* transmission. Future research should explore the role of biosecurity measures and targeted interventions based on these risk factors to mitigate *S. Dublin* prevalence.

Key Words: *Salmonella* Dublin, biosecurity, calves

1409 Association between dam factors and *Salmonella* Dublin positivity in dairy calves in early life. C. M. Lyn^{*1}, A. L. Greer², Á. Abuelo³, S. J. LeBlanc¹, and D. L. Renaud¹, ¹*Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada*, ²*Department of Biology, Trent University, Peterborough, ON, Canada*, ³*Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI*.

The objective of this study was to evaluate the association between dam factors and the odds of a calf testing positive for antibodies to *Salmonella* Dublin (*S. Dublin*). A total of 460 calves were enrolled from 4

commercial dairies in British Columbia, Canada, all confirmed positive for *S. Dublin* through serial bulk tank milk samples. Calves were fed colostrum from either their own dam, stored colostrum from another cow, or colostrum replacer. Further, depending on the farm, calves were fed whole milk (pasteurized or nonpasteurized) or milk replacer. Blood samples were collected from calves at a mean ($\pm$ SD) of 3 ± 2 d of life. Milk and blood samples were collected concurrently from each dam at 11 ± 9 DIM. The PrioCHECK *S. Dublin* Strip Kit (Thermo Fisher Scientific) was used to analyze both milk and serum samples, with a cut point of 35% positivity. Transfer of colostral immunity was assessed in calf serum using a Brix refractometer. A mixed logistic regression model, with farm as a random effect, was used to evaluate the association between a calf's *S. Dublin* status and that of its dam. Due to inconsistent recording, colostrum source was not included in the analysis. With respect to calves, 24.5% were positive, whereas 12.3% of dams were positive in both milk and serum ELISA, 1.7% were positive only in

serum, and 40.2% were positive only in milk. For every 1% increase in Brix percentage in serum, the odds of *S. Dublin* positivity increased 1.53 times (95% CI 1.20–1.95, $P = 0.001$). Compared with calves from negative dams, the odds of *S. Dublin* positivity were 2.83 times greater (95% CI 1.54–5.17, $P = 0.001$) if the dam was milk ELISA-positive and 6.10 times higher (95% CI 2.84–13.11, $P < 0.005$) if positive on both serum and milk. Calf *S. Dublin* positivity was strongly associated with dam infection status, with higher odds observed in calves from milk ELISA-positive dams. The association between serum Brix percentage and *S. Dublin* positivity suggests a high likelihood of transfer of *S. Dublin* maternal antibodies. This may complicate the interpretation of serology results in young calves.

Key Words: colostral antibodies, disease transmission, neonatal immunity

Breeding and Genetics 2: Genetics of Novel Health, Fertility, and Welfare Traits

1410 Utilizing trimmer-recorded hoof lesions for a national hoof health genetic evaluation in the United States. K. L. Parker Gaddis^{*1}, A. S. Ling¹, R. H. Fourdraine², and J. B. Cole^{1,3}, ¹*Council on Dairy Cattle Breeding, Bowie, MD*, ²*Dairy Records Management Systems, Raleigh, NC*, ³*Department of Animal Science, North Carolina State University, Raleigh, NC*.

Hoof diseases causing lameness are the second most costly disease on dairy farms, typically leading to additional costs for treatment and in severe cases removal of affected cattle. A genetic evaluation for hoof health would provide dairy producers information that can be used for selection. A pilot study was conducted using on-farm records of lesions reported by hoof trimmers. A total of 721,209 lesion records were available, representing 830 herds across 37 states from 2002 to 2024 and including 15 different lesions. A total of 550,000 records were matched to lactation records in the National Cooperator Database. Variance components were estimated using both linear and threshold animal models for comparison. Two population subsets were also compared: one including only those animals that were seen by the trimmer (TRIM) and the other including all animals recorded as being in the herd at that time (ALL). Analyses were completed for the most frequently reported events including digital dermatitis (DD), ulcers (UL), abscesses (AB), and white line separation (WL). Broader categories were defined as any infectious lesion, any noninfectious lesion, or any lesion regardless of cause. Effects in the model were those included in national health evaluations: year-season of calving, age at calving by parity, random herd-year, random herd-sire, additive genetic, permanent environment, and covariates for inbreeding and heterosis. The incidence of any lesion was 9.4% in the ALL subset, compared with 26.2% in the TRIM subset. Threshold model heritabilities were greater than those estimated with a linear model, regardless of population subset. For the TRIM subset, heritabilities estimated with a linear model ranged from 0.8% (WL) to 6.3% (DD) and from 3.4% (WL) to 7.8% (DD) with a threshold model. Estimated heritabilities were slightly lower in ALL. Average traditional reliabilities from linear models ranged from 15% to 25% depending on the lesion, while both subsets reached maximum reliabilities >95%. Results indicate that these data could be used for routine genetic evaluations.

Key Words: hoof health, genetic evaluation, hoof lesion

1411 Unraveling the genetic architecture of udder conformation traits derived from Cartesian coordinates generated by robotic milking systems in American Holstein cattle. G. C. Medeiros^{*1,2}, J. B. S. Ferraz², L. P. B. Sousa Junior¹, A. Suárez-Vega⁴, V. B. Pedrosa¹, J. P. Boerman¹, S. Y. Chen³, and L. F. Brito¹, ¹*Department of Animal Sciences, Purdue University, West Lafayette, IN*, ²*Department of Veterinary Medicine, School of Animal Science and Food Engineering of the University of São Paulo, Pirassununga, SP, Brazil*, ³*Farm Animal Genetic Resources Exploration and Innovation Key Laboratory of Sichuan Province, College of Animal Science & Technology, Sichuan Agricultural University, Chengdu, Sichuan, China*, ⁴*Dpto. Producción Animal, Facultad de Veterinaria, Campus de Vegazana s/n, Universidad de León, León, Spain*.

The adoption of automated milking systems (AMS) has transformed dairy farming, highlighting the significance of udder conformation traits for improved efficiency, health, and welfare. This study aimed to estimate

genetic parameters and elucidate the genetic basis through GWAS for 5 AMS-derived udder conformation traits in American Holstein cows: rear teat distance (RTD), front teat distance (FTD), udder depth (UD), udder balance (UB), and distance front-rear (DFR). Daily milk yield (DY) and milk electrical conductivity (EC) were included to assess productivity and udder health. Data collected from Cartesian teat coordinates by 36 AMS robots for 4,480 cows produced a total of 5,317,488 records. Of these cows, 4,118 were genotyped, featuring 57,600 SNPs. A mixed linear model estimated SNP effects using the BLUPF90 software, with Bonferroni correction applied based on the expected number of independent chromosomal segments. The GALLO R package was used to detect genes within ± 60 Kb from the significant SNPs, and functional enrichment analyses were performed using the DAVID platform. The heritability estimates for UB, UD, FTD, RTD, and DFR ranged from 0.41 to 0.79, with repeatability exceeding 0.80 for all traits. Moderate genetic correlations were found between some trait pairs, such as FTD with RTD (0.54) and UD with DFR (−0.47). Favorable correlations between DFR and DY (0.30) and UD and EC (−0.25) suggest the feasibility of multitrait selection. The GWAS identified 52 significant SNPs across 17 chromosomes, with genes associated with immune response, inflammatory pathways, transcriptional regulation, and hormonal signaling. This study highlights the potential of AMS-derived data for genomic predictions, enabling fast genetic progress through genomic selection. Our findings can contribute to breeding programs aimed at improving udder conformation, productivity, and health in precision dairy systems, helping to ensure efficient milking in AMS herds.

Key Words: udder conformation from milking robots, genetic parameter, GWAS

1412 Colostrum yield is associated with cows' performance in subsequent lactation and udder conformation indexes in Italian Holsteins. A. Costa^{*1}, G. Visentin¹, A. Goi², R. Finocchiaro³, M. Marusi³, J. T. van Kham³, L. Benzoni³, M. De Marchi², and M. Cas-sandro^{2,3}, ¹*Department of Veterinary Medical Sciences, Alma Mater Studiorum, University of Bologna, Bologna, Italy*, ²*Department of Agronomy, Food, Natural resources, Animals and Environment, University of Padova, Padova, Italy*, ³*Associazione Nazionale Allevatori della Razza Frisone, Bruna e Jersey Italiana (ANAFIBJ), Italy*.

Quick administration of good quality colostrum is pivotal for neonatal calves to ensure passive transfer of immunity. Although either colostrum yield (CY) or quality of dams is sometimes insufficient, farmers bypass this issue by drawing from the internal colostrum bank, administering fresh colostrum of other cows, or using preparations enriched with Ig, making colostrum individual traits rather neglected phenotypes. Our aims were to compare lactation performance of Holstein cows with different levels of CY at first milking and assess correlations with official genomic indexes calculated by Italian National Association of Holstein, Brown and Jersey Breeders (ANAFIBJ) in early 2025. Colostrum yield data referred to 2022 and 61 farms of Northern Italy. For each cow ($n = 2,280$), we had CY volume recorded within 6 h from calving along with milk yield (MY, kg) and protein content (PC, %) at first test-day (15–60 DIM), MY at lactation peak, and average MY and PC in the first 150 DIM. For the ANOVA, the model included farm as random, whereas parity, season of calving, IgG concentration as covariate, CY class (low, <3 L; medium-low, 3–4 L; medium-high, 4–6 L; high, >6 L),

and interaction between parity and CY class were fixed effects. MY at first test-day, at lactation peak, and its 150-d average were significantly affected by CY and the greatest LSM always in the high class. Least squares means of PC revealed an opposite trend. For example, PC at first test-day was $3.20\% \pm 0.24\%$ and $3.09\% \pm 0.26\%$ in the low and high CY class, respectively. The ANAFIBJ indexes were available for 215 cows genotyped in 6 out of the 61 initial farms. The aggregate udder index was significantly associated with CY (-0.28) and raw means were 0.09 in low class and -0.80 in high class. Conformation indexes were significantly correlated with CY too: overall morphology (-0.22) and udder support (-0.24), depth (-0.26), and attachment (-0.27). Our findings open the debate on the possibility to develop a selection index for dams' "colostrability" based on CY and Ig concentration for improving calves' resistance to diseases, and survival.

Key Words: calf health, immunoglobulin, selective breeding

1413 Advancing dairy calf health: Integrating diarrhea and respiratory health information into US national genetic evaluation. M. Neupane¹, K. L. Parker Gaddis², S. Toghiani¹, A. M. Miles¹, J. Graham¹, J. F. Burchard², J. W. Dürr², J. B. Cole^{*2}, J. R. O'Connell³, C. P. Van Tassell¹, and P. M. VanRaden¹, ¹*Animal Genomics and Improvement Laboratory, Agricultural Research Service, USDA, Beltsville, MD*, ²*Council on Dairy Cattle Breeding, Bowie, MD*, ³*University of Maryland School of Medicine, Baltimore, MD*.

Dairy calf health is a critical factor in the sustainability and profitability of dairy farming. Calf diarrhea (DIAR) and respiratory illnesses (RESP) are leading causes of calf mortality. This study aimed to develop a comprehensive US national genetic evaluation for these important calf health traits using producer-recorded data from the National Cooperator Database. Analyses included 207,602 calf records for DIAR (3–60 d of age) and 681,741 records for RESP (3–365 d of age) from all breeds (~97% data from Holstein and Jersey calves) born between 2013 and 2024. Both traits were modeled as binary variables (0 = diseased, 100 = healthy). The overall incidence rates were 14.46% for DIAR and 16.05% for RESP, with Jerseys having a higher incidence than Holsteins. The median incidence age was 10 d for DIAR and 68 d for RESP. Heritability estimates from the all-breed model were 0.025 for DIAR and 0.021 for RESP. Reliabilities of genomic predicted transmitting abilities (PTA) were higher than those of traditional PTA, and young sires (born after 2020) had high reliability estimates, highlighting opportunities for genetic improvement in calf health. Holsteins exhibited higher genomic reliabilities for both traits compared with Jerseys, likely due to the larger number of genotyped and phenotyped animals. A moderate genetic correlation (0.22) was observed between DIAR and RESP, while correlations with heifer livability, another calf health trait currently evaluated, were 0.13 for DIAR and 0.35 for RESP. Correlations of these health traits with other traits were low. These findings demonstrate the feasibility of including DIAR and RESP in the US national genetic evaluation system, offering a valuable tool for breeders to enhance calf health and survivability through genomic selection.

Key Words: genomic selection, calf, health

1414 Introduction of calf health traits into Canadian genetic evaluations for Holsteins. C. Lynch^{*1}, J. Jamrozik^{1,2}, A. Fleming¹, F. Miglior^{1,2}, C. F. Baes^{1,2}, and B. J. Van Doormaal¹, ¹*Lactanet Canada, Guelph, ON, Canada*, ²*Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada*.

The dairy industry faces the challenge of improving calf health while maintaining and enhancing animal health standards. The 2 most prevalent calf diseases on Canadian farms are respiratory problems (RESP) and diarrhea (DIAR). Genetic selection is one tool that can help reduce their incidence. Lactanet Canada has developed a genetic evaluation for calf traits, with official evaluations to be available for the Holstein breed starting August 2025. Lactanet Canada also intends to incorporate calf traits into both national selection indexes, namely the Lifetime Performance Index (LPI) and Pro\$, in April 2026. Producer-recorded calf disease data were used for the evaluation. After quality control, the evaluation test-run included 316,932 Holstein calf disease binary records for RESP and 130,027 for DIAR across 1,217 Canadian dairy herds from 2007 to 2024. The overall prevalence for RESP was 19.5% and for DIAR was 21.1%. A 2-trait single-step genomic evaluation with a linear animal model was conducted using MiX99 software. The model factors included a fixed year-season effect, and random herd-year-season, animal, and residuals. The reference population i.e., genotyped animals in the 4-generation pedigree included 107,254 animals. Heritabilities of 0.054 for RESP and 0.044 for DIAR, with genetic correlation of 0.526 between the traits were previously estimated in the same population by MC-EC-REML. For both traits, 2,974 sires achieved official status based on minimum herds, daughters and reliability, and over the years, no genetic trend of sires was observed, indicating the need for direct selection on calf disease traits. Estimated breeding value proof correlations between calf disease traits and other currently evaluated traits indicated only very weak relationships ($< \pm 0.15$), further emphasizing the need for direct selection on calf disease traits. For inclusion in national evaluations, Lactanet Canada is proposing an index weighting of 30% on RESP and 70% on DIAR, driven by economics, mortality rates, and the role of DIAR as a major precursor to RESP.

Key Words: genetic evaluation, calf health

1415 Genetic relationships between estrus expression traits and production traits in lactating dairy cows. J. A. Chasco^{*1}, R. S. Bisinotto², R. C. Chebel², K. A. Weigel¹, and F. Peñagaricano¹, ¹*University of Wisconsin–Madison, Madison, WI*, ²*University of Florida, Gainesville, FL*.

Dairy cow estrus is marked by acute changes in behavior, such as increased activity and decreased rumination. These changes can be measured on the farm using wearable sensors and other monitoring systems. The main goal of this study was to estimate the genetic variability of estrus expression traits and their genetic correlations with production traits in lactating dairy cows. Data consisted of bi-hourly activity and rumination records collected on 8,800 dairy cows using an automated monitoring device during 2 yr on a large commercial farm. Changes in activity and rumination were quantified to identify and characterize estrus events during the voluntary waiting period (11–70 DIM). Alternative estrus expression traits were evaluated, including estrus count, duration, and strength. Production traits included average milk production in the period 11 to 70 DIM (MY) and also 305-d mature equivalent milk production (305ME). Heritabilities and genetic correlations were estimated using bivariate animal models including year-season and lactation as fixed effects and cow as a random effect. The heritability of estrus expression traits ranged between 0.15 ± 0.02 (counts), 0.18 ± 0.02 (duration) and 0.29 ± 0.03 (strength), while the heritabilities of production traits were 0.34 ± 0.02 for MY and 0.43 ± 0.02 for 305ME. Estrus count was negatively correlated with both MY (-0.28 ± 0.08) and 305ME (-0.26 ± 0.08), indicating the high-producing cows tend to show less estrus events in the voluntary waiting period. On the other hand, neither estrus duration nor strength were genetically correlated

with production traits. Overall, estrus expression traits are heritable. Of special interest, the number of estrus events, but not its duration or strength, is negatively correlated with milk production.

Key Words: estrus, production, genetic correlation

1416 Genetic parameters for udder conformation and milking temperament in US Holstein cows derived from automatic milking system. P. Khanal*, P. Ross, and N. Deeb, *STgenetics*.

Automated milking systems (AMS) collect extensive data on each milking session, providing an objective approach to evaluate udder conformation and milking temperament without subjective human bias. Various studies suggest that AMS-recorded connect time (CT) and udder conformation traits can serve as proxies for classifier-assessed temperament and udder conformation. This study aimed to estimate the heritability of the udder conformation and milking temperament traits using AMS data from US Holstein cattle. A total of 1,455,514 daily CT records and cartesian teat coordinates were collected from 7,308 multiparous lactating cows ($n = 1, 2, \text{ or } 3$) across 4 STgenetics research herds from 2019 to 2024. Five udder conformation traits were derived from teat coordinates: front teat distance (FTD), rear teat distance (RTD), distance front-rear (DFR), udder balance (UB), and udder area (UA). The statistical models for udder conformation traits accounted for fixed effects of location, lactation number and stage of lactation, and random effects of additive genetic, permanent environment and herd-year-month. For CT, model also included udder conformation as a covariate. Univariate and bivariate analyses were performed to estimate heritabilities and genetic correlations. Pedigree data were used to define genetic relationships and estimate variance components. Heritability estimates ($\pm$ SE) for UB, FTD, RTD, DFR, UA, and CT were 0.41 ± 0.02 , 0.67 ± 0.02 , 0.50 ± 0.02 , 0.48 ± 0.02 , 0.57 ± 0.02 , and 0.15 ± 0.01 , respectively. Genetic correlations ranged from 0.76 ± 0.03 (UA-RTD) to -0.16 ± 0.03 (UB-RTD) among udder conformation traits. Connect time was corrected for udder coordinates to ensure its independence from udder conformation, reflecting only temperament. The moderate to high heritabilities suggest genomic selection could improve these traits. Targeted selection in breeding programs may reduce cows with unfavorable teat and udder conformations, improving suitability for robotic milking. The results could be used to design selection index for precision dairy breeding and optimize genetic progress for AMS adaptation.

Key Words: automatic milking systems, udder conformation, milking temperament

1417 GWAS beyond SNPs: Identifying copy number variants associated with health and fertility traits in Holsteins. G. C. Ladeira*, P. Pinedo², J. E. P. Santos¹, W. W. Thatcher¹, and F. M. Rezende¹, ¹University of Florida, Gainesville, FL, ²Colorado State University, Fort Collins, CO.

Copy number variation (CNV) has been implicated in various fertility and disease phenotypes in humans and livestock species. After mapping CNVs from high-density SNP genotype intensity data using PennCNV, we conducted SNP- and CNV-based GWAS to detect genetic variants underlying clinical endometritis (CE, $n = 3,303$), metritis (MET, $n = 3,287$), and 305-d pregnancy status (P305, $n = 3,291$) in Holstein cows. Genomic quality control yielded 578,677 SNPs and 2,654 CNVs. Population structure was controlled by fitting a genomic relationship matrix built using 50,000 random SNPs, sampled proportionally to chromosome size. SNP or CNV effects were tested one marker at a time, alongside with random polygenic genetic effect and fixed categorical effects of

farm, year, season, and cow parity. Only categorical levels with at least 3 cases, 3 controls, and daughters from 3 different bulls were analyzed. The SNP-GWAS revealed one region on BTA4 associated ($P \leq 0.05$) with CE and P305, with 13 genes mapping within a 1Mb interval. *AGR2*, involved in inflammatory diseases in humans, and *AHR*, acting on ovarian regulation and pregnancy maintenance in humans and mice, were among these genes. A region significantly associated with P305 was flagged by SNPs on BTA10, harboring the *BUBR1* gene, implicated in fertility in mice. Genomic regions located on BTA6 and BTA19 showed suggestive SNP associations with MET ($P = 0.053$) and CE ($P = 0.051$), respectively. CNV-GWAS identified one region on BTA29 associated with CE and suggestively associated with MET ($P = 0.07$). Notably, this region harbors the *KDM2A* gene, involved in DNA repair, follicle development in mice, and immune tolerance in humans, emphasizing its relevance to pregnancy and immunity. Clearly, SNPs and CNVs reveal distinct genomic regions associated with pregnancy outcomes and reproductive-related diseases, underscoring their complementary roles in association studies. Consequently, integrating SNP- and CNV-based GWAS results provides a more comprehensive understanding of the genetic architecture underlying low-heritable traits.

Key Words: structural variation, low heritability, reproductive performance

1418 Multitrait genomic models with trait- and marker-specific weights can substantially increase the accuracy of genomic breeding values. T. Meuwissen¹ and V. Boerner*, ¹Norwegian University of Life Science, Ås, Norway, ²GHPC Consulting and Service Pty. Ltd., Armidale, NSW, Australia.

The genome-wide association-based best linear unbiased prediction (GWABLUP) approach used GWA analysis results to differentially weigh the SNPs in genomic prediction, and was found to improve the reliabilities of genomic predictions. However, the proposed multitrait GWABLUP method assumed that the SNP weights were the same across the traits. Here we extended and validated the multitrait GWABLUP method toward using trait-specific SNP weights. In a 3-trait dairy dataset, multitrait GWAS estimates of SNP effects and their standard errors were translated into trait-specific likelihood ratios for the SNPs having trait effects and posterior probabilities using the GWABLUP approach. This produced trait-specific prior (co)variance matrices for each SNP, which were applied in a SNP-BLUP model for genomic predictions, implemented in the APEX linear model suite. In a validation population, the trait-specific SNP weights resulted in more reliable predictions for all 3 traits. Especially, for SCC, which was hardly related to the other traits, the use of the same weights across all traits was harming genomic predictions. The use of trait-specific SNP weights overcame this problem. In a multitrait GWABLUP analyses of a 32,201 Norwegian red cow population, of which 30,213 animals were used for training and 1988 were used for validation, trait-specific SNP weights resulted in up to 13% (e.g., 0.199 to 0.226 for milk yield) more reliable genomic predictions than unweighted SNP-BLUP, and improved genomic predictions for all 3 studied traits.

Key Words: GWAS, ssGBLUP, reliability

1419 Mid-infrared spectroscopy: Exploring a novel approach to estimate genetic relationships in dairy cattle. C. Machefer*, F. Miglior^{1,2}, R. E. Jahnel¹, F. S. Schenkel¹, and C. F. Baes¹, ¹Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, ²Lactanet Canada, Guelph, ON, Canada.

The integration of high-throughput phenotypic data into livestock breeding programs offers new opportunities to improve selection efficiency. A new concept explored in plant breeding used spectral data as an alternative or complement to genomic selection, to infer relationships between individuals instead of molecular markers. In dairy cattle, the use of mid-infrared spectra (MIRS) from milk samples may provide an opportunity, as milk spectra are routinely available, reflect milk biochemical composition and may capture genetic information. We aimed to determine whether MIRS could be a substitute for genetic relationships by comparing a (hyper)spectral (H) relationship matrix with the genomic relationship matrix (G), and to assess the consistency of milk MIRS over time for applications in breeding programs. In this study, 113,822 milk samples from 2,354 multiple parities genotyped Canadian Holsteins were analyzed using mid-infrared spectroscopy (1,060 wavenumbers from 5,008 to 925 cm^{-1}). The MIRS data were reduced to 512 wavenumbers by removing water absorption noise. The H matrix was defined as $S \cdot S'$, where S was the centered and scaled matrix for each

wavenumber, divided by the number of wavenumbers. The comparison of H across lactation with G involved principal component analysis (PCA) of both matrices, Mantel test using 999 permutations and covariance matrix analysis, with sample eigenvectors assessing variation. The first 2 principal components of the PCA performed on G explained 17.4% and 4.9% of variation, while the PCA on H explained 76.2% and 22.2%, respectively. The genomic relationships (G) showed weak correlation with H (Mantel test: 0.09 and high differences between the 2 covariance matrices). Moderate consistency in H matrix was observed across lactation stages (Mantel test: 0.42 between start and end), with stronger correlations between adjacent stages (0.60 from start-middle and 0.65 from middle-end). The application of milk MIRS-based relationships in dairy cattle may include the prediction of individual animal performance, which has been rarely applied in livestock.

Key Words: relationship matrix, mid-infrared spectroscopy, dairy cattle

Dairy Foods: Products and Chemistry

1420 High protein, low viscosity? N. Piskors* and J. Hinrichs, *University of Hohenheim, Stuttgart, Germany.*

Fermented milk products, also referred to as milk protein-based microgel dispersions, with high protein (8%–12%) and low-fat contents, such as skyr, fresh cheeses, and concentrated yogurts, are becoming increasingly popular. However, these products are often not consumed because of very dry and firm textures. Furthermore, sensory defects, such as roughness and graininess, increase with higher protein contents. Strategies currently available to improve the textural properties of these products often have disadvantages. For example, mixing with yogurt lowers the protein content, and mechanical treatments may even lead to higher viscosities. Therefore, this study aimed to employ low-frequency high-power ultrasound as a post-treatment to tailor the textural attributes of a fat-free fermented concentrated microgel dispersion, namely, fat-free fresh cheese. A pilot-scale test set-up was developed to allow a continuous post-treatment with low-frequency ultrasound (20 kHz) of fresh cheese (9% and 11% [wt/wt] protein) at a fixed pressure and volume flow (0.15 MPa and 84 L/h), different temperatures (5–35°C), and energy inputs (0–140 MJ/m³). Apparent viscosity and yield stress were determined from oscillatory and rotational rheology measurements. Volume-based particle size distributions and volume fractions were obtained using static light scattering. With increasing energy input, the apparent viscosity, yield stress, and particle size of the fresh cheese decreased significantly ($P < 0.05$), while the polydispersity of the product was only slightly altered. The lower apparent viscosities and yield stress values are attributed to lower volume fractions of the microgel particles. Raising the treatment temperature to 35°C reduced yield stress and apparent viscosity significantly ($P < 0.05$) and promoted the formation of visible aggregates with $>100\ \mu\text{m}$. No significant changes ($P < 0.05$) in rheology were observed after storage for 2 wk at 6°C. As such, the application of low-frequency high-power ultrasound is a promising approach to achieve a spoon- to drinkable high-protein fermented milk product with a creamy appearance.

Key Words: ultrasound, volume fraction

1421 Rheological and functional properties of a whey protein phospholipid concentrate-based beverage. F. Jalil Mozhdehi¹, S. G. Larson¹, and G. Ozturk¹, *¹Department of Food Science, University of Wisconsin–Madison, Madison, WI, ²Center for Dairy Research, Madison, WI.*

Whey protein phospholipid concentrate (WPPC), a byproduct of whey protein isolate production, is a rich source of bioactive compounds, including choline, which supports brain function, hepatic metabolism, and cellular structure and signaling. Despite its importance, choline intake remains inadequate for most of the US population, with postmenopausal women particularly at risk of due to hormonal and metabolic shifts. Therefore, finding new strategies to increase choline intake becomes crucial to addressing the underconsumption of choline. This study developed a WPPC-based beverage and evaluated the rheological properties of its ready-mix powder including apparent viscosity, consistency coefficient (K), and color at pH native. Functional properties, including heat stability index, water-holding capacity, powder solubility, and sedimentation, were assessed at 3 pH levels (4.5, 7, and native) in triplicate. The compositional information of WPPC powder was 70.33% protein, 17.62% fat, and 3.22 mg choline/g. In the WPPC-based ready mix, each 46-g serving provided 21 g of protein. Results using one-way

ANOVA and Tukey's post hoc test indicated significant variation in heat stability across the 3 pH levels ($P < 0.05$), with the highest stability observed at native pH (96.4%) and the lowest at pH 4.5 (88.8%). Water-holding capacity was significantly higher at native pH (15.1%) than pH 7 (5.0%), while no significant difference was observed between pH 4.5 (11.7) and 7 or between native pH and pH 4.5. Sedimentation results showed a significant difference between 3 pH values ($P < 0.05$), with higher and lower sedimentation at pH 4.5 (20.0%) and pH native (6.7%), respectively. The solubility index was significantly lower at pH 4.5 (84.8%) compared with pH 7 (90.0%) and native (91.4%). The consistency coefficient (K) was found to be 466,761 Pa.sⁿ, showing a high apparent viscosity. Color analysis revealed L^* of 56.59 ± 0.7 , a^* of 4.78 ± 0.1 , and b^* of 9.08 ± 0.04 . These findings provide insights into the physicochemical behavior of WPPC-based beverages and highlight key considerations for optimizing their functional properties in food applications, ultimately establishing their potential health effects.

Key Words: choline, postmenopausal women, whey protein phospholipid concentrate (WPPC)

1422 Investigating the impact of thermal processing on the structure and oxidative changes of whey protein phospholipid concentrate. R. Goodman¹, M. T. Armstrong¹, F. Jalil Mozhdehi¹, L. McDougall², J. Rugji¹, A. Girard¹, M. P. Richards^{1,3}, and G. Ozturk¹, *¹Department of Food Science, University of Wisconsin–Madison, Madison, WI, ²Department of Integrative Biology, University of Wisconsin–Madison, Madison, WI, ³Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

Whey protein phospholipid concentrate (WPPC), a byproduct of whey protein isolate production, is a rich source of bioactive compounds, including milk fat globule membrane proteins and lipids; however, it remains underutilized. There is growing interest in further processing or fractionating WPPC to extract these valuable compounds, yet challenges arise due to extensive processing that includes multiple heat treatments, which may trap fats with the proteins. This study evaluates 2 pilot-scale WPPC trials derived from Cheddar cheese whey: one processed from raw milk with a single batch pasteurization (batch WPPC) and the other from HTST milk undergoing a second HTST treatment (2xHTST WPPC). The resulting 2xHTST WPPC underwent post-filtration heat treatments such as additional HTST treatment, spray drying, and retorting. Post-production analysis assessed how these treatments influence lipid and protein oxidation and structural properties. For lipid oxidation, Batch WPPC had the lowest peroxide value (PV) at 5.40 mmol LHP/kg lipid and the highest TBARS value at 0.68 mmol TBARS/kg lipid. In contrast, the 2xHTST, 3xHTST, spray dried, and retorted samples displayed significantly higher PVs but lower TBARS, suggesting that HTST treatment may stabilize against secondary oxidation products. SDS-PAGE analysis revealed larger protein aggregates in the 3xHTST and retorted samples, confirmed through MFG and dynamic light scattering measurements and confocal laser scanning microscopy. Fourier-transform infrared spectroscopy indicated notable differences in the secondary structure of 2xHTST WPPC proteins versus Batch WPPC proteins. β -LG content was quantified using HPLC, and showed that 3xHTST WPPC protein contained significantly less β -LG compared with protein found in Batch WPPC (28.54 vs. 40.90 mg β -LG/g protein). This suggests that thermal processing may lead to the unfolding and aggregation of β -LG. These findings underscore the substantial impact of thermal processing on the structure and oxidative changes of WPPC,

highlighting the need for further research to optimize its processing and fractionation for improved utilization.

Key Words: whey protein phospholipid concentrate (WPPC), heat treatment, lipid oxidation

1423 Linear and nonlinear rheological behavior of ice cream. M. A. U. R. Alvi* and S. I. Martinez-Montegudo, *New Mexico State University, Las Cruces, NM.*

The rheological behavior of ice creams formulated with varying protein ratio (whey protein/casein) in the linear viscoelastic (LVE) regimen and nonlinear (NL) regimen were investigated using small amplitude oscillatory shear (SAOS) and large amplitude oscillatory shear (LAOS), respectively. Three protein ratios (13/87, 20/80, and 59/41) were tested in formulations containing 4.5% total protein, 13% total fat, and 42% TS. Ice creams formulated with a 13/87 ratio exhibited a wider LVE and higher magnitude of storage module (G') than 20/80 and 59/41 (up to 2- and 8-fold, respectively). The storage and loss moduli (G' and G'') decreased with increasing the content of whey protein in an exponential fashion ($R^2 = 0.998$). The LAOS analysis indicated moderate nonlinear elasticity ($e_3/e_1 > 0$), with elastic Chebyshev coefficients ratios of 0.2661, 0.2089, and 0.0804 at a frequency of 10 rad/s and a strain rate of 1,000%, for the 13/87, 20/80, and 59/41 formulations, respectively. The 13/87 ratio had the highest degree of intracycle strain stiffening of 0.48 at 400% strain and 0.84 at 1,000% strain, suggesting an ability to recover from large deformations. Lissajous-Bowditch curves showed strain-thinning behavior for the 3 formulations. The confocal laser scanning microscopy was performed to obtain density measurement of fat and quantification of particle size and number for ice cream mixes and ice creams. The highest number of particles was for the 59/41 ice cream mix with 8,228 particles, and the highest density of fat was also found to be for the 59/41 ice cream mix at 0.122 ± 0.044 .

Key Words: large amplitude oscillatory shear, ice cream, whey protein/casein ratio

1424 Concept for microwave-assisted extrusion—Design, microwave tuning, feasibility study. F. Schmidt*, B. Graf, and J. Hinrichs, *University of Hohenheim, Stuttgart, Germany.*

In recent advancements in food processing, microwave heating has emerged as it provides fast, direct and volumetric heating without adding water or steam. In this study, 2 technologies - extrusion and microwave - were combined, and a microwave-assisted extruder ($m = 30 \text{ kg/h}$; $P_{MW,max} = 6 \text{ kW}$) was built. First, the extruder was completely filled with water and heated stationary at the microwave entry point at different tuner positions and distances between the tuners (positions 0–20 cm; gap 3–12 cm). The reflected microwave power and the increase in temperature were examined. Optimized tuner settings (position 12 cm; gap 10 cm) achieve the lowest possible reflected power ($<10\%$) with simultaneous intense heating. On this basis, a feasibility study was carried out with the model system cheese curd (pasta filata; pH 5.7; lactic acid; fat in DM 45%). The product outlet temperature and chemical composition were investigated at different screw speeds (10, 15, and 20 rpm) and microwave power levels (0.8–1.4 kW). The product temperature increased with increasing power consumption, while it decreased with increasing screw speed at constant power consumption. Product temperatures around 58°C for at least 45 s are necessary to achieve complete plasticization of the curd. The fat, protein, and DM content of the extruded raw cheese did not differ significantly (one-way ANOVA with subsequent Tukey test; $P = 0.05$) in any of the tests. The CLSM images showed that an anisotropic

structure of the pasta filata-style cheese was achieved. It was shown that a pasta filata-style cheese can be produced using microwave-assisted extrusion. Typically, extrusion applications are limited in upscaling, as adequate and uniform heat transfer and distribution are hindered by indirect heating as the volume of the extruder increases in the third power, while the surface area increases only quadratically. In the set-up presented, heating is carried out directly by microwaves, which in combination with the rotating screw, leads to increased heat transfer and, thus, to uniform heating of the product. Calculations have shown that upscaling to 2,000 kg/h is possible with microwave extrusion and that it is a practicable new process technology that still requires further research and is currently being researched.

Key Words: microwave, extrusion, cheese curd

1425 Probiotic yogurt modulates gut microbiota and improves glucose regulation in a type 2 diabetes-prone mouse model. H. Shi*¹, T. Jetton², and J. Kraft^{1,2}, ¹*Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT,* ²*Department of Medicine, Division of Endocrinology, Metabolism and Diabetes, University of Vermont, Burlington, VT.*

Probiotic yogurt has been reported to reduce the incidence of type 2 diabetes (T2D) by modulating gut microbiota. However, results vary across studies, as not all probiotic strains exhibit anti-diabetic effects. This study examined the impact of yogurt containing *Lactobacillus plantarum* KABP-051 and *Bifidobacterium* BB-12 on glucose metabolism and gut microbiota in T2D-prone mice. Leptin-deficient (B6 Lep^{ob/ob}) 5-wk-old male and female mice were fed either a standard (STD) diet or a high-fat, high-sucrose (HFHS) diet for 16 wk. Mice were randomly assigned to 1 of 3 groups: (1) no yogurt (CON-, $n = 20$), (2) standard yogurt (CON+, $n = 40$), or (3) standard yogurt containing 10^9 cfu/g of KABP-051 and BB-12 (PRO, $n = 40$). Mice received 1 g/d of their respective yogurt, and BW and feed intake were measured weekly. Fed blood glucose (BG) was measured monthly. At the end of the study, fasting BG and hemoglobin A1C (HbA1C) were determined, followed by fecal sample collection for microbiota analysis. Yogurt consumption did not affect BW and feed intake, regardless of diet. On the STD diet, PRO yogurt lowered fed BG ($P = 0.002$), fasting BG ($P = 0.016$), and HbA1C ($P = 0.007$) compared with CON-. Although neither CON+ nor PRO affected fed BG or HbA1C on the HFHS diet, both improved baseline fasting BG ($P = 0.011$, $P = 0.054$, respectively). Although diet and yogurt treatment did not affect α -diversity, they altered β -diversity (Bray-Curtis, Jaccard, $P = 0.001$). Compared with CON-, PRO yogurt increased the phylum abundance of *Actinobacteria* and decreased *Firmicutes* in both diet groups. Furthermore, PRO yogurt decreased *Proteobacteria* and *Verrucomicrobiota* on the STD diet but increased *Verrucomicrobiota* on the HFHS diet (all $P < 0.05$). The HFHS diet raised the *Firmicutes/Bacteroidetes* (F/B) ratio by 45% compared with the STD-fed groups ($P = 0.046$). Importantly, in both diet groups, PRO yogurt reduced the F/B ratio by 46% compared with CON+ ($P = 0.013$). In conclusion, PRO yogurt improved glucose homeostasis by reducing fasting BG and HbA1C, and altered gut microbial composition, suggesting potential benefits in alleviating T2D symptoms through gut microbiota modulation.

Key Words: dairy, glucose homeostasis, gut microbial composition

1426 A refined approach for optimizing oligosaccharide recovery from bovine colostrum. K. Klein*¹, J. Renaud², A. J. Fischer-Tlustos¹, M. A. Steele¹, and A. Marciniak³, ¹*Department of Animal Biosciences, Guelph, ON, Canada,* ²*London Research and*

Bovine oligosaccharides (OS) are bioactive compounds that are found at higher concentrations in colostrum compared with whole milk, suggesting their potential importance for neonatal gut function. However, due to the complexity of colostrum composition, their fractionation and purification remains challenging. The objective of this study was to optimize the purification and recovery of OS from bovine colostrum and evaluate the effectiveness of recovering OS from colostrum whey permeate (WP) versus whey. The method is adapted from previous research which involved defatting colostrum and coagulating caseins to produce whey. In the first condition, whey was ultrafiltered using a 10 kDa membrane to obtain WP. Then lactose hydrolysis (LH) and monosaccharide fermentation (MF) were performed followed by ultrafiltration using a 150 kDa membrane. The second condition consisted of performing LH and MF directly on whey, before the 10 kDa ultrafiltration step. Lastly, both products were concentrated using a 600- to 800-Da nanofiltration membrane. Oligosaccharide concentrations (3'sialyllactose, 6'sialyllactose, 6'sialyllactosamine, and disialyllactose) at each recovery phase were measured using high-resolution MS. In the final retentate after nanofiltration, the second method showed higher OS purity (13.4% vs. 10.9% [% DM]) and recovery (17.3% vs. 3.7% [% DM]) compared with the first method. Results demonstrate that performing LH and MF directly on the whey rather than WP yields a higher purity and recovery of total OS. The refinement of techniques for OS recovery were improved compared with the first condition tested and indicates future opportunities to further optimize the process, enabling greater utilization of these bioactive compounds for neonatal gut health applications.

Key Words: colostrum, oligosaccharides, membrane filtration

1427 Development of a single-gene approach to subtype dairy-associated *Microbacterium*. Y. Deng*, C. Loomis, and N. H. Martin, Cornell University, Ithaca, NY.

Microbacterium (*M.*) is an emerging bacterial contaminant in the dairy industry, known for being thermotolerant, ability to form biofilms, and enzyme production, which can compromise dairy product quality. Here we develop an effective subtyping method and characterize high-risk *M.* strains relevant to dairy processing environments. A single-gene subtyping approach using *rpoA* (α -subunit of RNA polymerase) was developed and evaluated for discriminating between closely related *M.* species. We sequenced *rpoA* from *M.* isolates ($n = 296$) and identified 64 unique allelic types (ATs). To validate the accuracy of *rpoA*-based classification, whole-genome sequencing (WGS) was performed on a subset of dairy-related isolates. To compare *rpoA*- and WGS-based phylogenies, a randomized accelerated maximum likelihood tree and core genome SNP-based maximum likelihood tree were reconstructed

using *rpoA* sequences, and whole genome sequences, respectively, from 84 isolates. Identity score matrices were generated using clustering algorithms, where species groups were defined by bootstrap values exceeding 70% and percent identity scores above 97%. Overall, the 64 ATs were identified as belonging to 5 species groups, including *M. lacticum*, *M. paulum*, *M. oxydans*, *M. liquefaciens*, and *M. foiorum*. Comparative analysis showed strong agreement between *rpoA*- and WGS-based phylogenies, with major species groups and smaller clades clustering similarly. These findings support *rpoA* sequencing as an effective subtyping method to discriminate *M.* species into major taxonomic groups. Ongoing phenotypic evaluations under dairy-relevant conditions to assess strain-specific growth, biofilm formation, heat resistance, and enzyme production will further define the risk of dairy product contamination with diverse *M.* strains. These findings will help the dairy industry monitor, track, and mitigate high-risk *M.* strains across raw milk, processing equipment, and finished products.

Key Words: *Microbacterium*, dairy microbiology, subtyping

1428 Nanobubble technology for dairy processing effluent treatment. M. S. Mohan*, South Dakota State University, Brookings, SD.

Dairy processing effluent treatment breaks down the high organic load wastewater from dairy manufacturing processes, ensuring the quality of the effluent for discharge to surface waterbodies. Diversity in product portfolio in dairy plants causes huge variability in the organic load in the influent streams and thereby affecting the treatment efficiency and effluent quality. Aeration is a major step in effluent treatment ensuring the removal of total suspended solids (TSS) and the oxidation of dissolved solids, and air nanobubble treatment increased the dissolved oxygen levels to up to 51% in the treated wastewater compared with traditional aeration technology. We observed significant reduction in TSS, chemical oxygen demand (COD) and biological oxygen demand (BOD) values with air nanobubble treatment as compared with untreated model wastewater ($P < 0.05$), respectively. The evaluation of the nanobubble interactions with milk proteins in diluted systems indicated controlled aggregations of caseins as opposed to whey proteins, observed from the turbidity increase by 30% for the nanobubble-treated sample compared with the untreated samples. This indicates that one of the mechanisms for the reduction of TSS in wastewater streams is owing to controlled aggregation of proteins. The application of air nanobubbles for effluent processing in a commercial cheese and whey ingredient manufacturing company also indicated reduction in the TSS, COD, and BOD values of up to 25%, 10%, and 21% compared with the untreated wastewater, respectively. Overall, nanobubbles show great potential as a sustainable, low-cost and environmentally friendly technology for improving the efficiency of effluent treatment for dairy manufacturing plants.

Key Words: bubbles, aeration, wastewater

Milk Protein and Enzymes Symposium: Value-Added Dairy Powders with Specialized Functionality

1429 Caseinates: From bucket chemistry to specialized nutrition. T. Huppertz^{*1,2}, ¹*FrieslandCampina, Amersfoort, the Netherlands*, ²*University College Cork, Cork, Ireland*.

In milk, casein is primarily found in the form of casein micelles. However, in the manufacture of caseinates, the micellar structure is disrupted during the acidification and washing process, which removed the micellar calcium phosphate. On neutralization, casein particles are formed, the size, hydration, and other properties depend strongly on the degree of neutralization and the specific cations of the alkali used therefore. Caseinates of monovalent cations, such as Na or K, consist of defined hetero-oligomers of 10 to 12 caseins. Such particles have excellent heat stability and contribute primarily to viscosity by their nonspherical, elongated shape and water-binding. Caseinates of divalent cations, such as Ca, also contain such casein hetero-oligomers, but next to that also aggregates thereof. Viscosity is lower than for sodium caseinate due to reduced water-binding as a result of Ca-binding by caseins and less elongated shapes. Calcium-binding, and the concomitant charge neutralization, can also affect heat stability of caseinates. Overall, salts are critical in determining the properties of caseinates and are not only determined by the type and level of alkali, but also by residual salts in the washed acid-casein curd. These are primarily residual counterions of caseins and strongly determined by both the type of acid used for the acid-induced precipitation of the caseins as well as the pH at which precipitation is carried out. Although initial uses of caseins were often in nonfood applications, such as glues and paints, and later made an entry into basic foods for their water-binding properties and emulsification properties, they have since emerged as critical ingredients in many nutritional products, including medical nutrition products and protein bars. In medical nutrition, particularly Ca-caseinates play a critical role by providing a low-viscous, heat-stable protein sources with a complete amino acid composition that can also be used in conjunction with other protein sources. In protein bars, careful moderation of protein interactions via protein charge is critical to ensure optimal texture formation.

Key Words: milk protein, caseinate, functionality

1430 Multilength scale evaluation of dairy powders, reconstitution, and characteristics of recombined gel structures. K. G. Malmos^{*1}, T. V. Sønderby¹, A. Afrough³, P. Koch², P. Andersen², X. Qi³, C. Gaiani⁴, T. Vosegaard², S. Bakalis³, and M. Corredig⁵, ¹*Arla Foods amba, Aarhus, Denmark*, ²*Inter disciplinary nano science center (iNANO), Aarhus University, Aarhus Denmark*, ³*Department of Food Science, Copenhagen University, Copenhagen, Denmark*, ⁴*Laboratoire d'Ingénierie des Biomolécules (LIBio), Université de Lorraine, Nancy, France*, ⁵*Department of Food Science, Aarhus University, Aarhus, Denmark*.

The spray-drying process is pivotal in the production of dairy powders, designed to remove water while preserving product integrity. We assess the reversibility of water removal by comparing processability of liquid feeds and their respective powders. Although the dehydration process itself is reversible, predrying modifications such as diafiltration induce significant changes in the mineral content. We utilized ³¹P SS-NMR as a novel approach to probe the dynamics of phosphorus containing casein components indicating diafiltered caseins to have increased mobility. These predrying interventions lead to modifications that persist through drying and impact the final product characteristics. Additionally, we

investigated the crystallization behavior of lactose during the shelf life of dairy powders. Our findings indicate that lactose can migrate to the surface, forming crystalline structures that adversely affect the dissolution rate of the powder. We show that incomplete dissolution of powder ingredients results in poor product characteristics (i.e., water retention and brittleness) of acidified gels, whereas the slower process of mineral equilibration between serum and micellar state has limited effect on gel characteristics. This research underscores the importance of understanding predrying modifications, reconstitution state and modifications during storage to optimize the quality and functionality of spray-dried dairy products used for recombined products.

1431 Understanding the functional performance of milk powders. J. C. Brain^{*}, *Fonterra Research and Development Centre, Palmerston North, New Zealand*.

Reconstitution performance is a key feature of many dairy powders from commodity milk powders through to high-value protein powders. Test methods that measure aspects of powder reconstitution often lack the granularity required to drive process improvement and are often designed to replicate the conditions present in a given product application without thought to providing insights into the mechanisms driving functional performance. Fonterra have been involved in the development of several novel test methods that dramatically improve the objectivity and granularity of powder reconstitution tests. These new methods also have the flexibility to allow modification of test protocols to generate data that provides better insights into the mechanisms driving reconstitution performance. Understanding the mechanisms driving reconstitution performance and how they are impacted by the structure and chemistry of the powder can be useful in building insights into how to tailor formulation and processing conditions to target desired functional outcomes. Conditions in the spray dryer are well known to have a strong impact on the solubility of milk powders, with the rate of formation of insoluble material being a function of both concentration and temperature, peaking by orders of magnitude at the intermediate moisture contents that the product experiences as it transits the dryer. Products containing emulsified fat droplets have been shown to be significantly more susceptible to adverse drying conditions than those containing little or no fat, with the volume of insoluble material generated in whole milk powder being significantly higher than for skim milk powder under the same drying conditions. The formation of clustered fat droplets has been the focus of research relating to cream properties, the main causes of which are heat induced aggregation of proteins on the surface of fat globules and shear-induced formation of "homogenization clusters." The generation of homogenization clusters have been implicated in the increased insolubility of whole milk powder. Building on these insights, Fonterra is improving our understanding of the mechanisms governing milk powder reconstitution, helping to improve performance of existing products and inform development of new products.

Key Words: milk powder, reconstitution, performance, mechanistic understanding

1432 Functionality of high-protein milk powders in specific applications. V. Sunkesula^{*}, *Idaho Milk Products Inc., Jerome, ID*.

Milk protein concentrates (MPCs) are widely used in high-protein dairy formulations due to their excellent nutritional value and functional properties. They provide a high-quality source of protein, making them essential for products such as protein-fortified beverages, yogurts, and dairy-based nutritional supplements. However, despite their benefits, MPCs present significant functional challenges, particularly in high-protein applications. Poor solubility, increased viscosity, and heat-induced aggregation lead to undesirable textures, phase separation, and reduced product stability. These issues arise due to strong protein-protein interactions, inefficient hydration, and structural modifications during processing, limiting their broader commercial application. Researchers are developing advanced solutions to address these challenges and enhance MPC functionality. Mineral modification helps minimize

aggregation and enhance heat stability, while enzymatic treatments modify protein structures to improve solubility and gelation. Optimizing filtration techniques to alter MPC composition, such as casein to whey protein ratio, would improve their functionality and performance in some applications. Additionally, innovations in emulsification, water-binding, and whipping properties expand MPC applications in dairy and protein-based foods. These advancements enable the production of high-protein dairy products with improved quality, consumer appeal, and market potential.

Key Words: milk protein concentrate (MPC), high-protein food, functionality

Donald C. Beitz Recognition Symposium: 50-Plus Years of Dairy Science Research

1433 Don Beitz and colleagues' contribution to our understanding of transition cow hypocalcemia and other mineral challenges and strategies for prevention. J. P. Goff*, *Iowa State University, Ames, IA.*

Shortly after becoming a faculty member at Iowa State University (ISU), Don Beitz started working on all problems of transition cows. One of those problems involved hypocalcemia in dairy cows. Beitz collaborated with colleagues and students at Iowa State on this problem and began a long and fruitful collaboration with the Metabolic Disease Unit at the USDA, ARS, National Animal Disease Center (NADC) as well. The original low calcium diets for prevention of milk fever were performed at Iowa State University. How those diets worked to prevent milk fever was discovered through determination of blood parathyroid hormone concentrations and blood 1,25-dihydroxyvitamin D concentrations. The role phosphorus had on vitamin D metabolism was also discovered in the ISU-NADC collaborations, including experiments demonstrating milk fever could be controlled by reducing diet phosphorus. Various experiments outlining the role of vitamin D in periparturient mineral metabolism were conducted as well as trials testing various vitamin D compounds for their ability to prevent hypocalcemia and milk fever. Iowa State University and NADC became well known for these studies, and the work continues to day.

Key Words: hypocalcemia, milk fever, transition cow

1434 Advances in understanding fatty liver and ketosis from the work of Don Beitz and his colleagues. J. K. Drackley*, *University of Illinois Urbana-Champaign, Urbana, IL.*

Fatty liver and ketosis are 2 interrelated conditions affecting periparturient dairy cows that are associated with economic losses for the dairy industry. Don Beitz formed an early collaboration with his colleague J. W. Young at Iowa State University, which Beitz continued after Young's retirement. Early work sought to establish in vivo models of ketosis, using a combination of phlorizin treatment (to stimulate glucose loss in urine) and 1,3-butanediol (a ketone body precursor) in steers. Results showed that many metabolic aspects of lactation ketosis could be reproduced in steers. This led to a model in which fatty liver and ketosis were induced in early lactating cows by modest feed restriction (to 80% of energy requirements) coupled with supplementation of 1,3-butanediol. Several studies sought to refine the model and characterized various aspects of cows subjected to the model, including metabolites in blood and liver, glucose kinetics, and gene expression. A major finding in subsequent studies was that injections of glucagon could prevent and treat fatty liver in periparturient cows. Studies characterized this action of glucagon on metabolites and gene expression. Recent collaborations of Beitz have moved into in vitro models of fatty liver. Beitz's many contributions on ketosis and fatty liver have been foundational for other scientists to continue research into periparturient metabolism and physiology of dairy cows.

Key Words: hepatic lipodosis, ketosis, glucagon

1435 Advances in understanding bovine immunology from the work of Don Beitz and his colleagues. C. D. Nelson*, *University of Florida, Gainesville, FL.*

Don Beitz mentored several students with his colleagues at the USDA National Animal Disease Center whose focus was bovine immunology. He and his students advanced the understanding of how the plane of nutrition of growing calves altered immune status. For example, their work showed that feeding calves to achieve a growth rate of 1.2 kg/d compared with 0.55 or 0 kg/d resulted in diminished lymphocyte responses but stronger monocyte responses. Another contribution of Beitz and his students was the characterization of immune changes that occur in cows upon infection with *Mycobacterium avium* subspecies *paratuberculosis*. Several of his students evaluated the effects of vitamins A, D, and E on bovine immunity using cell-culture and animal experiments. Collectively, their work provided a framework for the mode of action of vitamins in bovine immune cells and a practical foundation for vitamin nutrition of cattle.

Key Words: immunity, vitamin, dairy cattle

1468 The legacy of Don Beitz and his passion for research and teaching and all things dairy, nutrition, biochemistry, and life. M. J. VandeHaar*, *Michigan State University, East Lansing, MI.*

Don Beitz is a distinguished professor of agriculture at Iowa State University with joint appointments in the Departments of Animal Science and Biochemistry-Biophysics. Don grew up on a farm in southern Illinois and earned his BS and MS degrees from the University of Illinois. He then earned his PhD in dairy nutrition and biochemistry from Michigan State University in 1967. Upon graduation, he began his career at Iowa State and never left. His extraordinary career spanning more than five decades has been marked by significant contributions to research, teaching, and service in the fields of animal science and biochemistry. In addition to the areas highlighted by other speakers, Beitz has advanced our understanding of milk fat synthesis, the genetic control of body composition, and the role of diet in human health and cholesterol homeostasis. He has published 300 peer-reviewed manuscripts, taught 350 courses to 16,000 students, and mentored 107 graduate students as major professor as well as numerous undergraduates. Don has been active in ADSA since 1964 and served as president in 2008 to 2009. He has been a staunch supporter of women and minorities in science, and maintained ties to former students with his annual Christmas letters now for 58 years. Don has seen and been part of many changes in dairy science over his career, but some things remained constant: his enthusiasm for basic science and its application, his ability to inspire a love of learning, and his faith and love for family and community. He has been a great example for many of us.

Ruminant Nutrition Platform Session: Alternative Feed Ingredients for Dairy Rations

1436 Of cows and bugs: Using insects as alternative feeds in dairy cattle nutrition. E. Rico*, *Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA.*

The steady growth of the human population and the reduction in the number of people in extreme poverty have significantly increased the global demand for animal foods. Protein shortages are a current threat to global food security, prompting us to reduce food waste, and to lessen the competition for protein sources between humans and food-producing animals. Although dairy cows typically consume feedstuffs not in direct competition with human food (e.g., forage, co- and byproducts), some others, such as soybean products still constitute an important source of high-quality protein in their diets. The use of alternative protein sources in dairy cow nutrition may help enhance their integration into circular economies, leading to more sustainable food production. Although the nutritional composition of insects can vary depending on species, rearing methods, life stage, and diet, these animals constitute potentially important sources of key nutrients for farmed animals, such as high-quality protein, lipids, and micronutrients. Although the Western Hemisphere is largely reticent to the incorporation of insects in the human diet (i.e., neophobia), its acceptance for the sustainable feeding of food-producing animals appears to trend positively. In this context, the industrial production of insects for animal feeding has risen quickly in the past few years, and it is expected to continue growing in the next decades. This matches the anticipated increment in the use of insects to replace conventional proteins of animal and plant origin in industries such as aquaculture, livestock, and pet food. Although very limited data are currently available on the impact of incorporating insect meals in dairy cows, *in vitro* studies, and others in small ruminants, indicate the potential for methane mitigation and no negative effects on animal performance. Our current work is exploring the feasibility of using insect larvae from the black soldier fly, as a partial substitute for soybean meal in diets for high-producing dairy cows. This presentation will explore the potential opportunities and limitations of using insects in the diets of dairy cows, while highlighting current gaps in knowledge and future work.

Key Words: dairy cow, insect feeding, sustainability

1437 Alternative foodstuffs in dairy ruminant nutrition: Basic concepts, recent issues, and future challenges. L. Pinotti*, M. Manoni¹, M. Ottoboni¹, and M. Tretola², *¹Department of Veterinary and Animal Science, Università degli Studi di Milano, Milan, Italy, ²Agroscope, Posieux, Switzerland.*

The sustainability of animal production is a major challenge in modern food production systems. Farm animals compete for nutrients and for the use of scarce resources, such as land, water, and energy, which are also essential for human nutrition. Addressing this competition is part of the ongoing debate at the scientific and public level. The goal is to produce more food, feed and energy globally without further burdening the limited availability of natural resources, particularly water and land. It is thus essential to redesign the concept of nutrition. One promising approach is to explore synergies between human and animal nutrition. Former foodstuff products (FFPs) and insects represent 2 examples of promising strategy for reducing feed-food competition through partial

replacement of grains and concentrate feed in ruminant diets without negatively affecting animal productivity and well-being. Although FFPs are rich in simple sugars and fats, excessive intake by ruminants may increase the risk of subacute rumen acidosis and modify bacterial microbial protein synthesis and methane emissions. Additionally, chemical substances present in FFPs (e.g., theobromine), and packaging remnants may alter ruminal function, milk production, and animal health. In the case of insects, the scenario is still ongoing, as in many countries, insect-based materials are not allowed as feed ingredients for ruminants. Furthermore, the stability of true proteins in the rumen is not so well defined, which complicates the definition of the possible rumen bypass of these matrices. As a result, farmers, nutritionists, industry, and governments are required to pay serious attention to the production processes of animal feed and to ensure the correct use of these materials in the target animals.

Key Words: dairy cow, alternative feed, quality, safety

1438 Alternative feed for livestock: Opportunities and challenges to support a circular food system. Z. Dou*, S. Rassler¹, and C. Jiya², *¹University of Pennsylvania, Kennett Square, PA, ²Lilongwe University of Agriculture and Natural Resources, Lilongwe, Malawi.*

The global livestock industry is at a crossroads to meet demand for animal-based food while simultaneously addressing sustainability challenges. Demand for meat, milk, and eggs is projected to increase by 60% to 70% by 2050. Much of the increase will come from developing countries due to increasing population, rising income, and urbanization. The heightened demand has increased requirements for livestock feed and other resources; however, feed production has been associated with deforestation, biodiversity loss, unsustainable resource extraction, and in particular climate change. Livestock-related emissions are estimated to contribute 11.1% to 19.6% of anthropogenic greenhouse gases, with a substantial portion originating from feed production and provision. Additionally, feed accounts for up to 60% of all farm expenses and has significant implications on livestock productivity. In this context, producing more meat, milk, and eggs for human consumption with fewer unintended consequences on the planet requires all workable solutions. Developing alternative feed is a viable option. Alternative feed is broadly defined as biomass materials that can be fed to livestock to support maintenance and production requirements, while improving agrifood system efficiency, contributing to the circular bioeconomy, and limiting competition of natural resources with human food production. This presentation will describe the nutritional attributes and feeding values of selected plant-based alternative feed ingredients. Two case studies will be presented focusing on the use of alternative feed in dairy production. The first will outline lessons learned from substituting culled fruit waste for conventional feedstuffs in modern dairy operations, and how collaboration with food supply chain stakeholders is integral throughout the process. The following case study will describe using human unfit biomass materials to support smallholder dairy operations in the Global South. These and other emerging opportunities, ingredient sourcing, and potential challenges to adopting alternative feed for enhanced sustainable livestock future will be reviewed.

Key Words: alternative feed, dairy, sustainable livestock

Production, Management, and the Environment Symposium: The DairyUp Project—Attacking Major Challenges for Dairy in a Multidisciplinary Project

1444 Case definition and metabolic disorders: More accurate phenotypes. I. J. Lean^{*1,2}, H. M. Golder^{1,2}, D. Sheedy^{1,2}, C. Old³, and A. Lean^{4,2}, ¹Scibus, Camden, NSW, Australia, ²Dairy UP Program, Camden, NSW, Australia, ³A3 Cattle Company, LeGrand, CA, ⁴School of Agricultural, Environmental and Veterinary Sciences and the Gulbali Institute, Charles Sturt University, Wagga Wagga, NSW, Australia.

Our objective is to more accurately define phenotypes of metabolic disorders. A lack of precision and clarity in defining metabolic disorders results in suboptimal progress in understanding, prevention, and treatment of these. Nondifferential errors in classification for dichotomous disorders result in weaker associations and drive hypotheses toward the null; differential errors result in unpredictable outcomes. For continuous variables, misclassification can result in differential errors; the degree and direction of this varies with the exposure distribution, category definitions, measurement error distribution, and the exposure-disorder relationship. Implications for definitions of feed efficiency (FE), ruminal acidosis, and lameness, and genetic progress are considered. Efficiency of beef and dairy cattle has been estimated by residual feed intake (RFI) based on residual estimates from a model predicting DMI as a function of ADG and BW^{0.75}. Residuals derived from the model include true variation in FE, but variation from a latent variable, composition of tissue gain, that is protein, fat, and other reserves that are gained and maintained with different efficiencies. Differences in RFI ranking of beef cattle for this model and others that include estimated change in body composition exist. In dairy cattle, inconsistencies exist between the RFI rank of heifers and cows. Genetic selection based on flawed RFI models raises the risk of selection for less fit genotypes. Ruminal acidosis is a prevalent disorder of cattle, but its temporal nature and the heterogeneity of the rumen both within and between days limits the diagnostic value of pH alone. Definitions not solely based on ruminal pH offer a potential for better phenotypic characterization and selection of more fit genotypes possibly improving production and health. Lameness has a low heritability; however, this is not a single disorder and evaluating these conditions as such raises the potential for differential and nondifferential error. Data provided to assess lameness are characterized by under-reporting and also have temporal challenges. DairyUP structures will and have been designed to address challenges of disorder definition to more accurately define phenotypes.

Key Words: feed efficiency, genotypes, ruminal acidosis

1445 Do cows in total mixed ration systems have old lipid profiles compared with pasture-based cows? D. B. Sheedy^{*1,6}, H. M. Golder^{2,6}, S. C. Garcia^{1,6}, Z. Liu³, P. Reddy^{3,4}, P. Moate⁵, S. J. Rochfort^{3,4}, J. E. Pryce^{3,4}, and I. J. Lean^{2,6}, ¹School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Camden, NSW, Australia, ²Scibus, Camden, NSW, Australia, ³Agriculture Victoria Research, AgriBio, Centre for AgriBioscience, Bundoora, VIC, Australia, ⁴School of Applied Systems Biology, La Trobe University, Bundoora, VIC, Australia, ⁵Melbourne, VIC, Australia, ⁶Dairy UP Program, Camden, NSW, Australia.

Disease and herd removal risks increase with parity, yet the underlying biochemistry of aging in cows is unknown. Feeding system may affect lipid profiles, but the consequences on aging and subsequent health is unknown. The objective of this cross-sectional study was

to explore associations of plasma lipids with age, parity, and feeding system (TMR vs. pasture-based [PB]). Plasma was collected from dry (n = 696, ~27 d prepartum), and peak-milk cows (n = 796, ~58 DIM) in a stratified (parity: 1, 2, 3, >3) random sample from 15 PB and 15 TMR farms. A targeted liquid chromatography-MS approach quantified 185 lipid species, including phospholipids, sphingomyelins, and triacylglycerols. Dry and peak-milk cohorts were analyzed separately. Machine learning algorithms identified lipid classes stably associated with age and parity (multiple linear regression, partial least squares [PLS], random forest [RF], support vector machine), and feeding system (sparse logistic regressions, PLS, RF). Older and greater parity cows were associated with low concentrations of phospholipids with the long-chain polyunsaturated omega-3 (n-3) fatty acids (FA) of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Dry PB cows had greater phospholipids containing α -linolenic acid (ALA, 18:3 n-3) and ALA derivatives than TMR cows. Peak-milk TMR cows had increased phospholipids containing linoleic acid (LA, 18:2 n-6) and its derivatives than PB cows. The n-3 FA are linked to improved immune function, cattle health, reproduction, and milk quality, while n-6 FA are pro-inflammatory. Importantly, both ALA and LA are essential FA that cannot be synthesized by the body and must be provided in the diet. These findings suggest that TMR cows experience a comparatively low n-3, high n-6 lipid environment, resembling an older cow lipid profile. We speculate that the different FA profiles of pasture (high ALA, low LA) and maize silage (low ALA, high LA) contribute to this observation. The different contributions of pasture and maize silage in diets of PB and TMR farms could theoretically have unintended consequences on cattle health and longevity.

Key Words: n-3 fatty acids, lipidomics

1446 The enteric and respiratory viral diversity of calves in health and disease—A state-wide metatranscriptomic study. B. P. Brito^{*1,4}, Z. U. Abedien^{2,4}, and I. J. Lean^{3,4}, ¹New South Wales Department of Primary Industries and Regional Development (DPIRD), Elizabeth Macarthur Agricultural Institute, Menangle, NSW, Australia, ²Australian Institute for Microbiology and Infection, University of Technology Sydney, Ultimo, NSW, Australia, ³Scibus, Camden, NSW, Australia, ⁴Dairy UP Program, Camden, NSW, Australia.

Despite extensive diagnostic efforts, many cases of respiratory disease and diarrhea in cattle remain unresolved due to inconclusive etiological findings. Untargeted metagenomics is a powerful approach for pathogen detection, enabling the comprehensive identification of microorganisms. In this study, we estimated the occurrence of viral species and genotypes in dairy farms across New South Wales, Australia. A total of 72 farms were sampled, with a proportional distribution across all dairy regions. Samples were collected from calves younger than 49 d. RNA was extracted from nasal and rectal swabs of 918 calves, and total RNA sequencing was performed on 408 nasal and 593 rectal swabs from calves with varying disease statuses. Additionally, a survey on calf management practices was completed by 52 farms. In total, at least 22 viral species and multiple genotypes were identified. To assess the association between viral presence and disease, transcriptomes were mapped to the identified viral genomes using Kallisto v0.5.1.1, and the relative abundance of viral reads was analyzed using DESeq2 v1.44.0 to compare diseased and healthy calves. The viruses most strongly associ-

ated with enteric disease were rotavirus and bovine kobuvirus, while bovine rhinitis virus A showed the strongest association with respiratory disease. Additionally, viral infections were significantly associated with specific age categories (0–7, 8–14, 15–42, and >42 d), particularly for some species of caliciviruses and picornaviruses. Viral detection was widespread among the 72 farms sampled, with kobuvirus present in 69 farms and bovine rhinitis virus A in 30 farms. We reconstructed the evolutionary relationships of 15 viral species for phylogenetic analysis, revealing substantial diversity in bovine rhinitis viruses A and B, bovine picornaviruses, and caliciviruses. Viral characterization at the genotype level enables a better understanding of the variants associated with disease. This level of resolution is critical for developing more sensitive diagnostic tools capable of distinguishing pathogenic strains.

Key Words: metagenomics, infectious diseases, calves

1447 Investigating genotype and environment effects on the rumen microbiome. H. M. Golder^{*1,2} and I. J. Lean^{1,2}, ¹*Scibus, Camden, NSW, Australia*, ²*Dairy UP Program, Camden, NSW, Australia*.

Currently there is a lack of capacity to rapidly and accurately detect rumen dysbiosis. Our objective is to improve phenotypic definitions of the rumen microbiome and metabolome to better utilize host and environment interactions to rumen challenges to enhance productivity. Our investigations of ruminal acidosis suggest that there would be an incidence of 500 cases over the first 100 DIM per 100 cows. There is marked among animal variation in response to typical and challenge environments. The task is to quantify the distribution of risk over time among cows given the temporal dynamic nature of the rumen environment and its microbiome, especially when perturbed. In a 293-cow multisite and -country GWAS, there was only a tendency for an association with being in the low-risk acidosis category. There were associations between the host and relative abundance of individual bacterial phyla and families and markers or tendencies for markers for acetate, butyrate, iso-butyrate, iso-valerate, and caproate. Others demonstrate some rumen microbes have a heritability of ≥ 0.15 . The rumen microbiome has substantial redundancy, which can produce inefficiency but offers protection against perturbation. Ruminants have a host-specific core microbiome, likely critical for basic function and a noncore probably for resilience. Investigation and understanding of the non-core may open greater potential for successful manipulation. Challenges in defining the rumen phenotype include what, where, who, and how often we should measure with high repeatability. We need to know whether some cows have nearly constant perturbation, while others exposed to the same diets are stable. Dairy UP is validating some potential markers and technologies from sensors, sniffers, artificial intelligence, camera surveillance, and bodily fluids at the individual and herd level to quantitate the lagged production responses and recoveries to dysbiosis. In summary if we don't appropriately define phenotypes of the rumen microbiome and metabolome we will not progress the field and optimize health and production.

Key Words: acidosis, dysbiosis, phenotype

1448 The positive associations between calf and lactation performances in dairy cattle. S. W. J. Legge^{*1,4}, P. C. Thomson^{1,3}, C. E. F. Clark^{1,2}, and S. C. García^{1,4}, ¹*Dairy Science Group, School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Camden, NSW, Australia*, ²*Sydney Institute of Agriculture, The University of Sydney, Camperdown, NSW, Australia*, ³*Sydney School of Veterinary Science, Faculty of Science, The University of*

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Australian dairy farms are increasingly capturing a wealth of data throughout the lifecycle of animals through the deployment of technology. This includes, among other data, details such as the sex of the calf, calving difficulty, health interventions, movement events, calving dates and parity, and milk production quantities. The underutilization of these extensive data presents a significant opportunity to optimize management practices, and drive improvements in productivity. The relationships between birth weight (BWT), weaning weight (WWT), lactation performance, and key production parameters have been explored in efforts to improve dairy cattle management. Our initial study demonstrated that there were potential indicators of high and low performance (measured as WWT) at very early age. Whether WWT relates to future lactation performance, both in terms of milk yield and number of lactations, remains unclear. Here, data from a cohort of 1,440 female Holstein Friesians were used to assess the impact of WWT (proxy for calf-rearing performance) on the number of lactations, milk yield per lactation and total lifetime milk yield. This study assesses how calf-related measurements might affect first lactation, lifetime production and age when animals are removed from production. Our results from modeling reveal that although BWT showed a slight positive linear trend with lactation-specific milk yield and a significant effect, the influence of WWT was far more pronounced with an additional 53.1 ± 8.5 kg milk yield/kg WWT in each lactation. Moreover, WWT exhibited a strong positive effect on lifetime milk yield and the likelihood of completing multiple lactations. Notably, we observed that calf performance at weaning significantly impacted the probability of remaining within the herd, where a calf with a WWT of 50 kg has a probability of 0.17 of attaining 3 or more lactations compared with 0.41 for a calf with a WWT of 100 kg. This has implications for overall lifetime productivity. These findings underscore the importance of early calf management and management decisions in optimizing milk production and herd sustainability.

Key Words: weaning weight (WWT), lactation performance, productivity

1449 Evaluation of the effects of an enzymatic and biological additive on bedding in compost-bedded pack barns in Australia. A. Lean^{*1,3}, H. M. Golder^{2,3}, J. Quinn¹, I. J. Lean^{2,3}, D. Sheedy^{2,3}, and A. Gunn¹, ¹*School of Agricultural, Environmental and Veterinary Sciences and Gulbali Institute, Charles Sturt University, Wagga Wagga, NSW, Australia*, ²*Scibus, Camden, NSW, Australia*, ³*Dairy UP Program, Camden, NSW, Australia*.

Bedding management in compost-bedded pack barns (CBPB) can be challenging. Maintaining a low-moisture and high-heat environment within the bedding is key to CBPB management. Manure Pro (Lallemant, Blagnac-Cedex) is a bedding additive we aim to assess for use in CBPB. A 5-wk exposure study was conducted on 40 areas in 5 CBPB in South-Eastern Australia from July 2023 to August 2024. Areas were monitored for bedding temperature at 10 to 25 cm, aerial ammonia (NH₃) and cow numbers within each area every 2 ± 1 d. Daily measures of external temperature, humidity and rainfall were recorded. Three farms were monitored for pack weekly moisture, carbon:nitrogen (C:N) levels and time cultivating the pack. The bedding was divided into treatment and control areas alternating along the bedded pack. Areas were monitored for 1 wk before treatment application at wk 2 and 4. Mean bedding temperatures per farm ranged from 19.29°C (SD 1.76) to 37.93°C (SD 3.42). Outcome means for control and treatment were bedding temperature 30.07°C (SD 7.37) and 30.09°C (SD 7.43),

percentage of cows per area 4.99% (SD 4.34) and 5.01% (SD 3.95), moisture 43.36% (SD 6.04) and 43.32% (SD 6.26), NH_3 0.03 ppm (SD 0.24) and 0.01 ppm (SD 0.15) and C:N ratio 12.47 (SD 1.39) and 12.71 (SD 1.59). Mixed models were used to evaluate the effects of treatment over time on bedding temperature, moisture, and cow preference with no changes ($P > 0.05$) being found. Ammonia was detected on 6 monitoring days over 3 farms. The farms evaluated for C:N were below the recommended 30:1, which is expected to cause increased microbial NH_3 production in the bedding and increased aerial NH_3 in the barn but this did not occur. There was no clear benefit of Manure Pro treatment in the study farms. A low C:N ratio may have led to reduced effectiveness in these CBPB. More studies to understand the interactions of Manure Pro with bedding should include cultivation time, moisture, C:N and controlled use of material additions.

Key Words: cow preference, ammonia, bedding temperature

1450 AI for a dairy cattle heat tolerance phenotype. A. Chlingaryan^{*1,2}, P. C. Thomson^{1,2}, S. C. Garcia^{1,2}, and C. E. F. Clark^{3,2}, ¹*The University of Sydney, Sydney, NSW, Australia*, ²*Dairy UP Program, Sydney, NSW, Australia*, ³*Charles Sturt University, Sydney, NSW, Australia*.

Climate change and extreme weather events pose substantial risks to dairy cattle production and welfare, with heat stress being a major disruptor. Current approaches for heat tolerance phenotype selection in dairy cattle primarily use statistical models. To enhance selection of heat-tolerant dairy cattle, we integrate AI with a statistical genetic

model that links heat tolerance to declines in milk, fat, and protein yield with temperature-humidity index (THI) rising above 60. This decline is modeled at both herd “population” and individual level, where the individual decline per unit increase in THI is represented by the corresponding random slope with the intercept providing the baseline. For this modeling, 20 yr of climate data were preprocessed and combined with New South Wales dairy cow herd test data for the Holstein breed, covering the same time period and resulting in 1.3 million records. Comparative analysis between the statistical and AI-based models demonstrated that the AI model had a smaller standard deviation of the residuals (3.25 L/d) compared with that for the statistical model (3.37 L/d). A high correlation ($R^2 = 0.90$) was observed between the random slopes estimated by both models, indicating that the models resulted in similar heat tolerance estimates but with a meaningful difference emphasized by the lower modeling error for the AI-based approach. A negative association between estimated random intercepts and slopes was identified, indicating that highly producing animals are less heat tolerant. Further analysis for heritability of the 2 traits (slope and intercept) using each method were done resulting in intercept: 0.354 ± 0.007 , and slope: 0.166 ± 0.006 for the AI model, and intercept: 0.362 ± 0.007 and slope: 0.140 ± 0.006 for the statistical model. Our findings highlight the importance of considering both traits to improve the selection for heat tolerance without compromising overall production levels. The proposed model combines the strengths of statistical modeling with the capabilities of AI, resulting in a more accurate and efficient means of identifying heat-tolerant cattle.

Key Words: heat stress, individual cattle level, milk production

Reproduction Symposium: Economics and Reproductive Efficiency in Dairy Farms—Simulation Models and Breeding Strategies

1451 Rethinking reproductive metrics: Stochastic models for economic and reproductive performance in dairy cows. V. E. Cabrera*, *University of Wisconsin–Madison, Madison, WI.*

Optimizing reproductive and economic performance in dairy herds is essential for achieving profitability and sustainability. This presentation highlights the innovative application of the Ruminant Farm Systems (RuFaS) animal module, a cutting-edge stochastic modeling tool that sets a new benchmark for evaluating reproductive programs. The RuFaS integrates cow- and heifer-specific decision making, simulating herd dynamics daily and assessing strategies such as semen selection, embryo transfer, and emerging technologies under diverse management and environmental conditions. Its modular design also supports additional assessments, including herd genetic progress and farm environmental impacts, making it uniquely suited for modern dairy systems analysis. Earlier stochastic approaches, such as Markov-chain models, laid a strong foundation for herd-level analyses tailored to farm-specific conditions. However, RuFaS advances these capabilities significantly, offering unparalleled customization for both whole-farm simulations and cow-specific decisions, such as precision breeding strategies. This granularity empowers producers to align individual animal management with broader farm goals, maximizing efficiency and profitability, while embracing precision agriculture techniques. Traditional reproductive metrics, such as the 21-d pregnancy rate, fall short of capturing the full economic consequences of reproductive performance. This work presents economic reproductive value (ERV) as a novel metric that integrates the speed and economic impact of pregnancies. The ERV combines costs (e.g., semen, labor, management) and benefits (e.g., milk production, calf sales, reduced culling) into a single, actionable measure. It shifts the focus from isolated reproductive events to comprehensive evaluations of herd and cow-level profitability, providing meaningful benchmarks for dairy producers. Findings from RuFaS simulations quantify how optimizing reproductive strategies enhances profitability, sustainability, and resilience, even under volatile market conditions. By enabling farm-specific analyses and precision decision making, RuFaS redefines benchmarks for reproductive success, delivering actionable insights for researchers, extension professionals, and dairy producers.

Key Words: stochastic modeling, reproductive efficiency, economic decision making

1452 Evaluation of early reproductive success prediction in commercial US dairies. B. Fessenden^{*1,2}, D. Liang², M. Borchers², D. Weigel², F. Di Croce², and M. Endres¹, ¹*University of Minnesota, St. Paul, MN*, ²*Zoetis Inc., Kalamazoo, MI.*

Reproductive performance impacts the profitability of a dairy farm. Culling risk, replacement heifer supply, and genetic progress are a few of the components of a dairy farm it impacts. The ability to understand the reproductive capabilities of individual animals and use of targeted reproductive management could optimize cow reproductive performance and herd profitability. To address this need, the early reproductive success prediction was developed using light gradient-boosting machine (LightGBM) algorithm incorporating 20 features encompassing cow performance, weather data, and herd-level reproduction features. The early reproductive success algorithm predicts the probability of pregnancy by 110 DIM. The objective of this study was to evaluate the ability of the algorithm to predict several reproductive performance metrics.

This retrospective study was conducted to demonstrate the ability, before the end of the VWP, of an early reproductive success prediction to predict pregnancy probability by 110 DIM. This study included 27,233 Holstein and Jersey multiparous cows that freshened in 2022 from 7 US commercial herds. Animals were ranked by their prediction within herd and then assigned to deciles based upon this ranking. Animals' performance data were collected for 18 mo following freshening from on-farm management software. Statistical analysis was performed using generalized linear mixed models with prediction decile as a fixed effect and herd and semen type as a random effect. Early reproductive success prediction deciles were different ($P < 0.0001$) for pregnancy at first AI (31.8% vs. 52.4%), proportion pregnant at 110 DIM (41.2% vs. 64.0%), and proportion of cows that gave birth to a live calf to initiate the following lactation, referred to as successful calf (58.4% vs. 81.3%), with percentage improvements in performance of 65%, 55%, and 39% respectively. This demonstrates that the early reproductive success prediction presents an opportunity for dairy producers to accurately identify the reproductive capabilities of individual animals and make informed management decisions.

Key Words: reproduction, precision livestock technologies, predictive algorithm

1453 Predictors of reproductive outcomes in seasonal calving pasture-based lactating dairy cows. E. M. Sitko* and S. T. Butler, *Teagasc, Animal and Grassland Research and Innovation Center, Moorepark, Fermoy, Co. Cork, Ireland.*

The objective of this retrospective observational study was to evaluate associations between predictor variables and reproductive performance outcomes in seasonal calving pasture-based dairy cows. Data were collected for 8,859 lactations across 21 herds in 2022 and 2023. Data included genomic proofs, calving and insemination records, milk production, and health and estrus alerts generated by automated activity monitoring systems (AAM; SenseHub, MSD Animal Health). Cow records were removed if (1) calving occurred outside the spring window (December to May), (2) artificial insemination (AI) service records were incomplete or first AI occurred outside the spring window (April to July). The final dataset contained 6,967 lactations. Binary data were analyzed using logistic regression, continuous data using linear regression, and time to pregnancy using Cox's regression. Parity (1, 2, 3, ≥ 4), number of estrus alerts before mating start date (EA; 0 vs. ≥ 1), fertility index (FI; H, M, L), number of health alerts within 70 DIM (HA; 0, 1, ≥ 2), and semen type (sex-sorted vs. conventional) were included as explanatory variables, and herd was a random effect. Pregnancy per AI at first service and days open (DOPN) differed ($P < 0.01$) for all explanatory variables evaluated. Cows with ≥ 1 EA had greater P/AI and fewer DOPN (51% and 23 d) compared with cows with 0 EA (37% and 33 d). Cows in the H-FI group had greater P/AI and fewer DOPN (54% and 26 d) compared with the M-FI (48% and 28 d) and L-FI (34% and 32 d) groups. Cows with ≥ 2 HA had reduced P/AI and greater DOPN (43% and 29 d) compared with cows with 0 HA (48% and 27d) and cows with 1 HA were intermediate (45% and 29 d). The hazard of pregnancy was greater ($P < 0.01$) for cows with ≥ 1 EA compared with 0 EA (1.9 [95% CI 1.7–2.1]). The hazard of pregnancy was greater ($P < 0.01$) for the H-FI group compared with the M-FI (1.2 [95%CI 1.1–1.3]) and L-FI groups (2.0 [95% CI 1.9–2.2]). The hazard of pregnancy was greater (P

<0.01) for cows with 0 HA compared with cows that had 1 (1.1 [95% CI 1.1–1.2]) and ≥ 2 HA (1.2 [95% CI 1.1–1.3]). Applying data predictive of reproduction outcomes could aid herd reproductive management.

Key Words: reproduction, seasonal calving, technology

1454 Maximizing farm profitability through breeding decisions. F. C. Ferreira^{*1}, J. M. Vieira², and M. I. Marcondes³, ¹*Clean Air Task Force, Boston, MA*, ²*Lawley's Inc, Tulare, CA*, ³*William H. Miner Agricultural Research Institute, Chazy, NY*.

Breeding strategies are an important management tool to maximize dairy profitability. Farmers can combine the use of conventional semen (CS), sexed semen (SS), beef semen (BS), and in vitro produced embryo transfer (IVP-ET) to design breeding schemes that will guarantee their supply of replacement heifers and to create an income stream from the sales of day-old dairy-beef crossbred (CROSS) calves. This decision-making process is dependent mainly on the reproductive performance of the herd and the market price of CROSS and pure beef calves. The goal of this work is to evaluate the economic feasibility of using different breeding schemes combining CS, SS, BS, and IVP-ET in Jersey and Holstein herds with 2 levels of reproductive performance, and different day-old CROSS and pure beef calves' prices. A Markov chain model can be used for this analysis by maximizing the profitability of dairy herds by changing the number of dairy heifers sold at birth and the culling rate of cows in the third or greater lactation. Under current scenarios in which day-old CROSS and pure beef calves have a high market value (varying from \$500–\$900), the use of BS in breeding schemes is warranted. The use of IVP-ET to produce pure beef calves, particularly in Jersey herds—where day-old male calves have little market value—can be a strategy to increase revenue, but profitability may vary. For instance, for Jersey herds using \$85 and \$170 IVP-ET to produce pure beef calves sold at 1 d old at \$300, profit/cow per year was reduced by 13% and 40%, respectively, compared with the combined used of BS and SS and the sale of day-old CROSS at \$110. The greatest economic opportunities are achieved in herds with good reproductive performance, in which more breedings can be directed toward using BS. Lastly, the management of surplus heifers is an essential economic consideration farmers should make. Raising surplus heifers has not been optimal due to their lower market price and high raising costs. However, under the current market scenario in which replacement heifers may reach \$4,000/head, a further analysis of the opportunity is warranted.

Key Words: breeding strategies, economics, opportunity

1455 Management strategies impacting pregnancy rate on Pennsylvania and Maryland dairy farms. S. Rassler^{*}, J. Ferguson, and J. Bender, *University of Pennsylvania, Kennett Square, PA*.

Despite evidence of efficacy, production efficiency, and financial incentive to highly regulate reproductive strategies, reproductive performance on dairy farms remains a significant opportunity for improvement. The objective of this study was to combine commercial dairy herd reproductive data with producer survey responses to identify dominant strategies resulting in high reproductive performance, as measured by pregnancy rate (PR). Herds utilizing DHIA services in Pennsylvania and Maryland were ranked by rolling herd average and categorized as high, medium, or low. Farms were ranked within each category and every other herd ($n = 283$) was selected for study inclusion. One hundred sixty-six farms returned surveys, a 59.7% response rate. Producer-reported data included practice of postpartum examinations, first and repeat service synchronization programs, and pregnancy diagnosis method(s). Repro-

ductive data for all active and culled cows calving in the previous 16-mo interval was accessed via DRMS (Raleigh, NC), imported into RepMon (University of Pennsylvania, Kennett Square, PA) and matched with survey responses. Records from 7 herds were incomplete and removed. Data were analyzed using MIXED procedure (SAS 9.4, $P < 0.05$) with herd size as fixed effect. Herds performing postpartum examinations tended to have lower PR (20.2%) compared with herds that did not (22.7%). Herds employing timed artificial insemination (TAI) without heat detection demonstrated the highest PR (25.3%). Presynch/ovsynch (PR = 25.7%) and G6G/ovsynch (PR = 26.9%) programs had higher PR compared with double ovsynch (20.8%) or ovsynch alone (21.4%). Repeat service TAI program did not influence PR; however, herds have an opportunity to improve PR by decreasing the interbreeding interval. Herds employing blood testing for pregnancy diagnosis had increased PR (25.9%) compared with milk testing (20.4%), transrectal palpation (19.7%), or transrectal palpation with ultrasound (22.2%). Findings from this study conclude that herds with high reproductive efficiency measured by PR omit postpartum examinations, employ presynch/ovsynch or G6G/ovsynch, and utilize blood testing for pregnancy diagnosis.

Key Words: pregnancy rate, reproduction, survey

1456 Effect of X- and Y-chromosome bearing sperm on conceptus attachment and pregnancy losses in lactating dairy cows. A. Santos^{*} and J. R. Pursley, *Michigan State University, East Lansing, MI*.

Differences in sex ratio at birth are most likely due to differences in fertilization rates or pregnancy losses favoring either X- or Y-chromosome-bearing sperm. This study was designed to assess the effect of X- or Y-chromosome-bearing vs. conventional semen on conceptus attachment (CA) and pregnancy losses of lactating dairy cows. We hypothesized that sorted X- and Y-chromosome-bearing sperm would differ in fertility from one another and would differ from the conventional batch from which they were collected when inseminated into lactating Holstein cows. Cows ($n = 884$) were synchronized with Double Ovsynch for first AI or GnRH + Ovsynch for second or greater AI. All cows received 1 mg of cloprostenol sodium at final PG. One technician performed all AI 16 h after the final GnRH. Cows were blocked by service sire and parity and were randomly assigned to receive conventional (CONV), X- (X), or Y (Y)-chromosome-sorted semen at AI. Each collection of semen from $n = 6$ Holstein bulls of high fertility and high net merit were divided into each of the 3 semen types. Only cows with confirmed ovulation to the final GnRH were utilized ($n = 264$ primiparous and $n = 589$ multiparous). Conceptus attachment was identified as a $\geq 10\%$ increase in serum pregnancy-specific protein B from d 17 to 26 post-AI. Pregnancy was diagnosed with ultrasound on 34 and 62 d post-AI. Data were analyzed in SAS 9.4 using the FREQ and LOGISTIC procedures. Differences in treatments were determined using $P < 0.05$. The X group had greater CA in primiparous cows (68%) compared with Y (54%) but not CONV (64%). In multiparous cows, CONV (65%) had greater CA compared with both X and Y (50 and 52%). The CONV group had greater P/AI on 34 and 62 d post-AI compared with X but not Y in multiparous cows only. Semen type did not affect pregnancy losses from CA to 62 d post-AI in primiparous (18% CONV, 15% X, and 8% Y) or multiparous cows (27% CONV, 30% X, and 22% Y). In conclusion, sperm sorting based on X- and Y-chromosomes affected conceptus attachment and pregnancy outcomes, but not consistently.

Key Words: conventional semen, X- and Y-chromosome-bearing sperm, Holstein

Ruminant Nutrition 4: Proteins and Amino Acids

1457 Bioavailability of rumen-protected Lys determined by continuous abomasal infusion of ^{15}N -Lys in lactating cows. J. Kim^{*1,2}, H. Hu², K. Park^{1,2}, and C. Lee^{1,2}, ¹*Interdisciplinary Nutrition Program, Department of Animal Sciences, The Ohio State University, Wooster, OH*, ²*Department of Animal Sciences, The Ohio State University, Wooster, OH*.

We determined the bioavailability (BA) of rumen-protected Lys (RP-Lys) using an isotope dilution method via ^{15}N enrichment of Lys in plasma or milk. Four ruminally cannulated mid-lactating cows were used in a 4×4 Latin square design with 19-d periods. The dietary treatments were CON (a basal diet meeting 89% Lys requirement), FLys (continuous abomasal infusion of 32 g/d free Lys on d 15 to 19), RPL1 (top-dress of 122 g/d RP-Lys 1; 65 g/d Lys), and RPL2 (top-dressed of 166 g/d RP-Lys 2; 65 g/d Lys). During sampling (d 15 to 19), cows received continuous abomasal infusion of 0.45 g/d ^{15}N -Lys. On d 18 and 19 in each period, urine, feces, and milk were collected via total collection and blood samples were obtained from the tail vein before feeding. Samples were analyzed for Lys concentration and ^{15}N enrichment in Lys. Digestible Lys flows (dLys) were calculated using the amount of ^{15}N -Lys infused and ^{15}N enrichment of Lys in either milk or plasma. Data were analyzed using the mixed procedure of SAS with random effects of cow and period, and a fixed effect of treatment. The ^{15}N enrichment of Lys was diluted in both milk and plasma ($P \leq 0.08$) for RPL1 and RPL2 compared with CON. However, the enrichment in milk Lys was similar, but that in plasma Lys tended to be different ($P = 0.10$) between RPL1 and RPL2. The least squares means of dLys for CON, RPL1, and RPL2 were used to calculate BA. The estimated dLys for control was 199 and 189 g/d when milk and plasma ^{15}N enrichments were used, respectively. The BA of RPL1 was 52% and 58% and the BA of RPL2 was 63% and 78% when calculated by ^{15}N enrichment of Lys in milk and plasma, respectively. In conclusion, dLys estimated for CON was comparable to that estimated by NASEM (183 g/d). The absolute BA of RPL1 and RPL2 were not in agreement between milk and plasma ^{15}N enrichment. The difference in ^{15}N enrichment of plasma Lys but no difference in that of milk Lys suggest that ^{15}N enrichment of plasma was likely less variable. However, the ranking of products was similar when BA was estimated from ^{15}N enrichment of milk or plasma Lys (RPL2 > RPL1).

Key Words: milk, plasma, stable isotope

1459 Effects of abomasal infusion of branched-chain amino acids or branched-chain keto-acids on circulating amino acids in blood plasma during early lactation. G. Ahmad^{*}, M. Sura, J. R. Daddam, K. Gallagher, I. Bernstein, and Z. Zhou, *Michigan State University, East Lansing MI*.

Fresh period is characterized by negative energy and protein balance. Previous results from our group showed that branched-chain amino acids (BCAA) infusion improved lactation performance whereas branched-chain ketoacids (BCKA) infusion reduced liver triglycerides in fresh cows, with favorable alterations in oxidative stress and inflammation during early lactation. The objectives of this study were to determine the effect of BCAA and BCKA on circulating amino acids in plasma during early lactation. Thirty-six multiparous Holstein cows were enrolled in a randomized complete block design at parturition. Cows were blocked according to expected calving date and were abomasally infused for 21 d after parturition with solutions of saline (CON, $n = 12$); BCAA

($n = 12$), including 67 g valine, 50 g leucine, and 34 g isoleucine; and BCKA ($n = 12$), including 77 g ketovaline, 57 g ketoleucine, and 39 g ketoisoleucine. All cows received the same diet. Blood samples were taken at 0, 3, 7, 14, and 21 d postpartum for quantification of 20 amino acids with LC-MS. Treatment effects were determined using PROC GLIMMIX in SAS. As expected, plasma BCAA concentrations were greater in cows receiving abomasal BCAA or BCKA infusion compared with CON ($P < 0.01$). Branched-chain amino acid infusion also increased plasma total AA (TAA; $P = 0.01$, 2,975.7 vs. 2,704.4, SEM 69.5) and EAA ($P < 0.01$, 1,227.3 vs. 846.3, SEM 48.5) compared with CON. Similarly, EAA also tended to be greater in cows receiving abomasal BCKA infusion during early lactation ($P = 0.06$, 980.6 vs. 846.3, SEM 48.9). Plasma sulfur-containing AA were lower in BCAA cows ($P = 0.01$, 134.2 vs. 146.1, SEM 4.4) and greater in BCKA cows ($P = 0.04$, 156.3 vs. 146.1, SEM 4.4) compared with CON, while urea concentrations were also greater in BCAA cows ($P < 0.01$, 4.46 vs. 3.82, SEM 0.16) compared with CON. Among individual AAs, BCAA infusion reduced alanine ($P = 0.04$, 219.6 vs. 242.7, SEM 11.1) and cysteine ($P = 0.01$, 115.7 vs. 127.9, SEM 3.2) compared with CON. Overall, the results indicate that abomasal infusion of BCAA/BCKA altered the plasma AA concentrations contributing to the changes observed during early lactation.

Key Words: early lactation, amino acid, ketoacid

1460 Effects of rumen-protected methionine on metabolism and lactation performance in dairy cows. W. M. Coelho Jr.^{*1}, M. J. Wollentarski¹, D. B. Melo¹, H. F. Monteiro¹, M. F. Vetter¹, M. F. Caballero¹, S. Olivarez¹, S. Locsin¹, E. Neto², J. Hergenreder², F. Sun², and F. S. Lima¹, ¹*Department of Population Health and Reproduction, University of California, Davis, Davis, CA*, ²*Kemin Industries Inc., Des Moines, IA*.

Supplementation with rumen-protected methionine (RPM) has been shown to enhance metabolism, milk yield, and milk components, but the responses are contingent on the encapsulation used. The study aims were to investigate the effects of RPM sources on metabolism and lactation performance. Holstein cows were blocked by parity and, within each block, randomly allocated to either CON (no RPM, $n = 15$), KST (KESSENT Me, Kemin Industries Inc., $n = 15$), or SMT (Smartamine, Adisseo, $n = 15$). Dry cows were enrolled at 245 d of pregnancy and fed individually in Calan gates. Cows in the KST and SMT groups received 5 g/d of RPM prepartum (27 ± 3 d) and 45 g/d postpartum (47 ± 3 d) to meet 100% metabolizable methionine. Cows had BW, BCS, blood, and milk sampling for solids performed weekly, and DMI and milk were measured daily. Data were analyzed using a mixed model or logistic regression on JMP. The fixed effects were treatment, parity, and the interactions treatment by parity, while the random effect was block nested within the parity group. There were no effects of RPM supplementation on BW in kg (KST = 641 ± 3.1 vs. SMT = 635 ± 3.1 vs. CON = 635 ± 3.1 , $P = 0.21$), BCS (KST = 2.95 ± 0.02 vs. SMT = 3.00 ± 0.02 vs. CON = 2.97 ± 0.02 , $P = 0.12$), prepartum DMI in kg/d (KST = 15.3 vs. SMT = 15.1 vs. CON = 15.8, $P = 0.34$), and postpartum DMI in kg/d (KST = 21.0 vs. SMT = 21.1 vs. CON = 21.0, $P = 0.99$). Among the plasma metabolites, only phosphorus tended ($P = 0.052$) to be higher in KST (6.4 mg/dL) and SMT (6.1 mg/dL) than in CON (5.5 mg/dL) in wk 1 postpartum. For lactation performance up to 50 DIM, the KST and SMT groups had a higher FCM in kg/d (KST = 48.1 ± 1.2 , SMT = 48.7 ± 1.2 vs. CON = 44.6 ± 1.1 , $P < 0.001$), ECM in kg/d

(KST = 46.5 ± 1.1 , SMT = 46.9 ± 1.1 vs. CON = 43.2 ± 1.1 , $P < 0.001$), and fat yield in kg/d (KST = 1.92 ± 0.05 , SMT = 1.97 ± 0.05 vs. CON = 1.76 ± 0.05 , $P < 0.01$) than CON cows, while no differences were found in protein yield ($P = 0.27$). Therefore, as hypothesized, RPM improves lactation performance, and the sources evaluated resulted in similar increases in FCM, ECM, and fat yield.

Key Words: energy-related metabolites, milk yield, transition period

1461 Effects of methionine analog on lactation performance during a heat stress challenge in postpeak lactating Holstein cows.

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This study aimed to evaluate the effects of methionine analog (METa; 2-hydroxy-4-(methylthio)butanoic acid [HMTBa] supplied as MFP feed supplement; Novus International Inc., Chesterfield, MO), fed at 0.24% of DMI, to provide a sufficient postruminal methionine supply, on lactation performance in postpeak lactating, multiparous Holstein cows during a heat stress challenge (HSC). Thirty-two multiparous, lactating Holstein cows (balanced by DIM [169 ± 66]; body surface area [6.73 ± 3.2 m²]) were randomly assigned to 1 of 2 dietary treatments: TMR with METa or TMR without (CON). Within each dietary treatment group, cows were randomly assigned to 1 of 2 environmental treatment groups in a split-plot crossover design. The study was divided into 2 periods with 2 phases each. In phase 1 (9 d), all cows were in thermoneutral conditions (TN) and fed ad libitum. In phase 2 (9 d), group 1 ($n = 16$) was exposed to a HSC using electric heat blankets. Group 2 ($n = 16$) remained in TN but was pair-fed (PFTN) to HSC counterparts. After a 14-d washout period, the study was repeated (period 2) and environmental treatments inverted relative to treatments from phase 2 of period 1, while dietary treatments (METa or CON) remained the same for each cow. Cows were milked 2 times/d, samples taken on d 1, 5, and 9 of each phase. Statistical analyses were performed using the MIXED procedure of SAS. There tended to be an environment $\times$ diet where cows in CON HSC had greater DMI than cows in CON PFTN, METa PFTN, and METa HSC (25.3, 22.9, 23.6, and 23.8 kg/d; respectively; $P < 0.01$). Cows in HSC had greater energy balance than PFTN (5.33 and 1.37 Mcal/d; respectively; $P < 0.01$). Cows in HSC had greater *longissimus dorsi* change from phase 1 than PFTN (-0.02 and 0.06 cm; respectively; $P < 0.01$). Cows in HSC had lower milk production (36.3 kg/d), fat concentration (3.65%), and protein concentration (3.10%) than PFTN (37.8 kg/d, 3.98%, and 3.21%; respectively; $P < 0.01$). In conclusion, cows exposed to heat stress had decreased milk production and greater protein mobilization. It is possible that rumen modulation from HMTBa during heat stress negatively affected DMI, leading to decreased milk production but without compromising energy balance or efficiency.

Key Words: 2-hydroxy-4-(methylthio)butanoic acid (HMTBa), heat stress, production

1462 Effects of rumen-protected His at different levels of metabolizable protein under adequate Met and Lys supply on nutrient digestibility and N utilization in lactating cows. K. Park^{*1}, J. Kim¹, H. Hu², M. Kambara³, K. Nakagawa³, and C. Lee², ¹*Interdisciplinary Nutrition Program, Department of Animal Sciences, The Ohio State University, Wooster, OH*, ²*Department of Animal Sciences, The Ohio State University, Wooster, OH*, ³*Ajinomoto Co. Inc., 1-1 Suzuki-Cho, Kawasaki-Ku, Kawasaki-Shi, Kanagawa, Japan*.

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

Previously, we found that various metabolizable protein (MP) diets with rumen-protected (RP) His did not affect performance when the requirement of Met and Lys were met. In the current study, we determined their effects on nutrient digestibility, N utilization, and plasma amino acids profile. Sixty mid-lactating Holstein cows were used in a randomized complete block design with the following diets: a MP-adequate diet that meets the MP, Met, Lys, and His requirements (AMP; CP, 17.0%); a MP-moderately deficient diet (92.6% of the requirement) meeting the requirement of Met and Lys (DMP; CP, 15.4%); the DMP diet with RP-His to meet the His requirement (DMPH); a MP-deficient diet (86.7% of requirement) meeting the requirement of Met and Lys (EDMP; CP, 14.5%); the EDMP diet with RP-His to meet the requirement of His (EDMPH) according to NASEM (2021). All samples were collected in the last week of the 8-wk experiment. Data were analyzed using PROC MIXED of SAS with a fixed effect of diet and random effect of block. Total-tract digestibilities of DM, OM, and NDF were not affected. Cows on AMP had greater total-tract N digestibility (69.6 vs. 64.1%; $P < 0.01$) compared with others while it was not affected by RP-His. Dietary treatments did not affect milk N secretion, but lowering MP supply linearly increased ($P < 0.01$) milk N / N intake and decreased ($P = 0.01$) urinary N excretion. RP-His supplementation decreased milk N / N intake ($P = 0.02$) because it did not affect N secretion in milk. Plasma His concentration tended to decrease (linear $P = 0.06$) as MP supply decreased but was not affected by RP-His supplementation. Carnosine concentration increased for EDMP and EDMPH ($P = 0.03$) compared with AMP without a difference between EDMP and EDMPH. In conclusion, lowering MP supplies under adequate Lys and Met increased N utilization for milk protein which was not further improved by RP-His supplementation. The increased plasma carnosine for EDMP and EDMPH suggests that His may have been provided from the endogenous pool and His deficiency did not occur.

Key Words: carnosine, milk nitrogen, His deficiency

Ruminant Nutrition 4: Lipids

1463 Dietary metabolizable protein and palmitic and oleic acids affect milk production in early lactation dairy cows. J. E. Parales-Girón^{*1}, J. de Souza², and A. L. Lock¹, ¹Michigan State University, East Lansing, MI, ²Perdue Agribusiness, Salisbury, MD.

We determined the impact of dietary metabolizable protein (MP) content and fatty acid (FA) supplementation on production responses of early-lactation cows. Ninety multiparous dairy cows were used in a randomized complete block design with a 2x3 factorial arrangement of treatments. Treatment diets were fed from 1 to 22 DIM and contained 2 levels of MP (low ~111 g/kg DM [LMP] and high ~122 g/kg DM [HMP]) and 0%, 1%, or 2% DM supplemental FA (Ca-salt containing 60% palmitic and 30% oleic acid). Treatment diets contained 30% NDF, 21% forage NDF, and 24% starch. From 23 to 50 DIM all cows were offered a common diet to evaluate carryover (CO) effects. The statistical model included the random effect of block, cow within block and treatment, Julian date, and the fixed effects of MP content, FA supplementation, time, and their interactions. We did not observe interactions between MP and FA for production responses (all $P > 0.60$). Compared with LMP, HMP increased the yields (kg/d; all $P < 0.01$) of milk (42.6 vs. 47.2), milk fat (1.90 vs. 2.11), milk protein (1.41 vs. 1.67), and ECM (48.8 vs. 55.2). Increasing supplemental FA (results presented in the following order: 0%, 1%, and 2% DM) increased (all linear $P < 0.05$) the yields of milk fat (1.91, 2.02, 2.09), milk protein (1.50, 1.55, 1.58), and ECM (50.4, 52.5, 53.2). After treatments ceased, we observed a CO effect with HMP increasing (all $P < 0.05$) the yields of milk (53.7 vs. 55.7), milk protein (1.53 vs. 1.61), and ECM (54.9 vs. 56.5). Increasing FA supplementation also had a CO effect, increasing (all linear $P < 0.05$) the yields of milk fat (1.99, 2.04, 2.10), milk protein (1.53, 1.57, 1.61), and ECM (54.6, 55.8, 56.7). In summary, dietary MP and supplemental FA (60% palmitic and 30% oleic acid) had additive effects on milk production. HMP+2%FA increased ECM yield by 8.9 kg/d compared with LMP+0%FA, while HMP vs. LMP and 2%FA vs. 0%FA increased ECM yield by 6.4 and 2.8 kg/d, respectively. These effects were observed during the immediate postpartum period and during peak lactation.

Key Words: early lactation, metabolizable protein, fatty acid

1464 Effect of a fatty acid supplement containing a blend of palmitic and stearic on nutrient digestibility and transfer efficiency of long chain fatty acids to milk. Y. Adeniji^{*} and K. J. Harvatine, Department of Animal Science, The Pennsylvania State University, University Park, PA.

Transfer efficiency of dietary fatty acids (FA) to milk is variable and not well understood. Our hypothesis was that increasing dietary FA would decrease transfer efficiency of preformed FA to milk fat by decreasing the direct transfer during the first 24 h after absorption. This was investigated using 8 mid-lactation ruminally cannulated Holstein cows (115 ± 32 DIM and producing 41.5 ± 4.43 kg of milk; mean ± SD) in a switch-back design with 21-d periods. Treatments were a low-fat basal diet (CON, total FA = 2.5%) and the basal diet supplemented with a blend of palmitic and stearic FA at 2% DM (FAT; 33.5% 16:0, 53.1% 18:0, and 6.16% 18:1 n-9). Data were analyzed with JMP Pro 18 with a mixed model that contained the random effects of cow and period and fixed effect of treatment with pretrial observation as a covariate for production data. Increasing dietary FA tended to increase milk yield by 2.2 kg/d and increased milk fat yield by 170 g/d ($P = 0.07$ and

0.02 respectively). Yield of >16C FA in milk was increased by 108 g/d with FA supplementation, largely due to increased 18C FA ($P = 0.01$). However, yield of <16C and 16C FA was not changed compared with CON. Apparent total-tract digestibility of DM, OM, NDF, and FA were not different. However, FA supplementation decreased apparent transfer of 18C FA from feed to milk fat (69% vs. 53%, $P < 0.01$). Transfer of absorbed 18C FA was 110% in the CON but decreased to 82% in the FAT ($P = 0.01$). On d 15 of each period, cows were abomasally infused with a free FA stock that provided 44 g of α -linolenic acid and 20 g of stearidonic acid and transfer kinetics monitored for 7 d. Total transfer of n-3 FA to milk fat was decreased by the fat supplement. The proportion directly transferred during the first 21 h did not differ between treatments and averaged 81%. Increasing dietary fat through FA supplementation did not affect digestibility and increased milk fat yield by increasing yield of preformed FA although preformed FA transfer efficiency was decreased.

Key Words: milk fat yield, perturbation model, preformed fatty acids

1465 Effect of feeding a calcium salt supplement containing omega-3 fatty acids during the transition period on production responses in early-lactation dairy cows. M. L. Miller, H. L. Reisinger, K. M. Gouveia^{*}, G. A. Contreras, B. J. Bradford, and A. L. Lock, Michigan State University, East Lansing, MI.

Our objective was to evaluate the effect of feeding eicosapentaenoic acid (EPA; C20:5 n-3) and docosahexaenoic acid (DHA; C22:6 n-3) during the close-up (CU) and fresh (FR) periods on production responses and metabolites in early lactation dairy cows. Thirty-five multiparous Holstein cows were used in a randomized incomplete block design. Treatments were a control diet containing a calcium salt of palm oil fed during the CU and FR periods at 0.60% diet DM (CON) or a diet containing a calcium salt enriched in EPA and DHA fed during the CU and FR periods at 0.90% diet DM (N3). Diets were balanced for dietary supply of palmitic, oleic, and linoleic acids. The CU period treatments were fed beginning 21 d before due date and FR period treatments were fed for the first 23 DIM. Cows were switched to a common diet from 24 to 63 DIM to evaluate carryover effects. The statistical model included random effects of block, cow nested within block and treatment, and Julian date, and the fixed effects of treatment, time, and their interaction. Results are presented in the sequence CON versus N3. Compared with CON, N3 during the CU period reduced colostrum yield by 33% (3.64 vs. 2.45 kg; $P = 0.05$). During the FR period, there was no effect of treatment on DMI, milk yield, BCS, or BW (all $P > 0.60$). Compared with CON, N3 reduced milk fat yield (2.12 vs. 1.94 kg/d; $P = 0.05$) and tended to reduce milk fat content (4.91% vs. 4.66%; $P = 0.10$) and yields of ECM (52.7 vs. 49.7 kg/d; $P = 0.10$) and FCM (53.1 vs. 49.7 kg/d; $P = 0.09$). Additionally, N3 reduced the yields of de novo (416 vs. 375 g/d; $P = 0.01$) and mixed (663 vs. 584 g/d; $P < 0.01$) milk FA sources. Plasma concentrations of insulin, glucose, NEFA, and BHB were not affected by treatment (all $P > 0.33$). During the carryover period, there was a treatment by time interaction as N3 reduced milk fat yield compared with CON for the first 2 wk on the common diet ($P = 0.10$). In conclusion, feeding a calcium salt enriched in EPA and DHA to dairy cows during the CU and FR periods did not affect milk yield but reduced colostrum yield and milk fat yield, which persisted through the first 2 wk of the carryover period.

Key Words: n-3 fatty acids, transition period, milk fat

1466 The short-term effect of increasing doses of palmitic and stearic acid on plasma fatty acid concentration and mammary arteriovenous difference in Holstein cows. A. N. Staffin* and K. J. Harvatiné, *Penn State University, University Park, PA.*

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

Palmitic acid (16:0) and stearic acid (18:0) are commonly fed to dairy cows but the effects of these fatty acids (FA) on circulating FA concentrations and mammary FA uptake are not well described. The objective was to use arteriovenous (AV) difference to quantify mammary FA extraction in cows fed increasing doses of supplements high in 16:0 or 18:0. The hypothesis was that 16:0 supplementation would increase mammary FA extraction more at lower doses compared with 18:0, and that both would reach a plateau within the doses tested. Twelve second-lactation Holstein cows (106 ± 31 DIM) were arranged in a replicated 3×3 Latin square design with a dose escalation design within each period and a ≥ 10 d washout between periods. Treatments included a no-supplement control (CON), a FA supplement high in 16:0 (PA; $>88\%$ 16:0 and 8% 18:1c9), and a FA supplement high in 18:0 (SA; 81% 18:0 and 10% 18:1c9). The FA supplements were fed at increasing doses every 4 d, targeting 0, 150, 300, 500, and 750 g/d. We previously reported that PA increased milk fat yield over CON starting at 300 g/d while SA only increased it at 750 g/d. Coccyeal vessel (representing arterial) and mammary vein samples were collected at 4 time points during the last 48 h of each PA and SA dose and composited before FA analysis by GC. Data were analyzed in a mixed model that included the random effects of cow and period and the fixed effects of treatment, dose, and their interactions. Preplanned contrasts compared PA and SA within each dose. Plasma 16:0 as a percent of total FA was enriched by PA and 18:0 was enriched by SA in both arterial and venous samples. Arterial concentration of 16:0 was increased starting at 150 g/d and total FA starting at 300 g/d with PA compared with SA, while there was no difference between PA and SA for 18:0 concentration. Plasma AV difference of 16:0 was greater for PA compared with SA starting at 150 g/d, but there was no effect of treatment on AV difference of 18:0 or total FA. Increased milk fat yield with PA occurs through an increase in arterial FA concentration and mammary uptake of 16:0.

Key Words: nutrition, lipid, metabolism

1467 Effects of dietary fatty acids on milk production and methane emissions in cows fed iso or non-isoenergetic diets. V. Ramos*, D. C. Reyes, V. R. Basnayake, M. R. Villalobos, B. M. Dulac, F. A. Gutierrez-Oviedo, L. A. Sprague, and J. W. McFadden, *Cornell University, Ithaca, NY.*

We investigated the effects of alternative fatty acid (FA) supplements in place of fiber (non-isoenergetic; NIE) or starch (isoenergetic; ISO) on milk production and ruminal gas emissions in cows fed a diet typical for California. Forty-eight multiparous Holstein cows (95 ± 21 DIM, 46.3 ± 6.47 kg of milk/d) were used in a split-plot 4×4 Latin square design with 21-d periods. Main plots were NIE and ISO diets containing 42% forage DM with nonforage aNDFom from almond hulls. Within each plot ($n = 24$), cows were assigned to 1 of 4 treatments, all supplemented with 2% FA (DM basis): (1) no supplemental FA (CON), (2) palmitic acid (PA; 85% C16:0), (3) calcium-salts of FA (CSFA; 59% C16:0, 25% C18:1, and 3% EPA/DHA), or (4) whole cottonseed plus canola oil (OIL). Model included treatment, plot, period, square, and treatment $\times$ plot as fixed effects. Dry matter intake, milk yield, and ECM yield were modified by treatment only (treatment; $P < 0.01$). In NIE plot, OIL increased milk fat content (4.08% vs. 3.60%; $P < 0.01$), relative to CON. In ISO plot, PA had greater milk fat content (4.49% vs. 3.85%; $P < 0.05$), relative to CON. In ISO plot, milk protein content was lower for all FA treatments ($\sim 3.33\%$; $P < 0.01$), relative to CON (3.50%). In NIE plot, milk fat yields were greater in cows fed OIL (1.82 kg/d), relative to all other treatments (~ 1.62 kg/d; $P = 0.02$). In ISO plot, milk fat yields were greater in cows fed PA (1.87 kg/d; $P < 0.02$), relative to CON (1.63 kg/d). In NIE plot, milk protein yields were unaffected by treatment. In ISO plot, milk protein yields were lower in cows fed PA and OIL (1.37 and 1.39 kg/d; $P = 0.04$), relative to CON (1.46 kg/d). In NIE plot, methane emissions were not modified by treatment. In ISO plot, FA supplements increased methane emissions (~ 381 ; $P = 0.05$), relative to CON (345 g/d). In NEI plot, hydrogen emissions were greater for PA cows, relative to all other treatments (1.43 vs. ~ 1.23 g/d; $P = 0.03$). In ISO plot, all FA supplements increased hydrogen emissions (~ 1.75 g/d; $P = 0.03$), relative to CON (1.41 g/d). Dietary FA supplementation modifies milk composition and gas emissions, with effects influenced by dietary energy density.

Key Words: fatty acid, energy, methane

ADSA Southern Branch Platform Session: Novel Approaches and Technologies for Monitoring Milk Quality

1500 Early mastitis detection using modern technologies on dairy farms. S. Paudyal*, *Department of Animal Science, Texas A&M University, College Station, TX.*

Mastitis remains a significant challenge in dairy farms, adversely affecting milk yield and quality, and increasing culling rates. Despite extensive research, mastitis prevention and treatment remain a major challenge in modern dairy farms. Traditional diagnostic methods, such as microbiological culture and SCC evaluation, involve milk-appropriate sampling and laboratory analysis. Although cow side tests have been available, they often lack the accuracy needed for reliable detection. The advent of modern tools and precision dairy technologies has revitalized the focus on mastitis detection. Early detection is crucial for minimizing the risk of chronic illness and enabling timely treatment and recovery. Daily monitoring of cow level parameters provides an opportunity to identify the deviations that are associated with the induction of mastitis events, thus presenting opportunities for early detection in subclinical stages. Numerous studies have explored various tools for this purpose. Although some tools directly monitor milk characteristics and components (e.g., electrical conductivity, somatic cells, and milk leucocyte differentials), technologies assessing cow health (e.g., rumination sensors, monitoring milk fat and protein components) are also extensively explored for their use in monitoring udder health. This presentation will summarize research findings on novel tools for monitoring udder health and detecting diseases in dairy cattle. As prevention and treatment of mastitis are dependent on the type of pathogen-associated, we further explore the opportunity and challenges associated with identification of pathogen groups causing mastitis events based on noninvasive parameters obtained in dairy farms. This will also set the stage for discussions on advanced analytics and the development of modern sensor and image-based tools for evaluating mastitis detection algorithms, which are continually evolving.

Key Words: mastitis, technology, detection

1501 Decades of infrared thermography research without farm applications: Why mastitis detection has not reached the real world, and why it still may. T. L. Wells* and A. R. Rivers, *United States Department of Agriculture, Gainesville, FL.*

Mastitis is a prevalent and economically significant disease in dairy cattle, leading to lower milk production, decreased milk quality, and increased treatment costs. Detecting mastitis early is crucial for effective treatment, but traditional diagnostic methods such as SCC testing and bacterial culture are expensive, time-consuming, and impractical for large dairy farms. For years, researchers have studied infrared thermography (IRT) as a noninvasive way to detect mastitis by measuring temperature changes in the udder caused by inflammation. More recently, artificial intelligence (AI) and deep learning (DL) have been used to analyze IRT images. However, despite decades of research, IRT is still not used in dairy production. We examine the approaches that have been taken to thermal imaging and why they have failed for reasons such as environmental factors, poor standardization, and spatial and interanimal variability. Past studies have also had statistical issues with experimental design, sample size, validation sets, and data leakage. This review looks at past research on IRT for mastitis detection and suggests ways to overcome these challenges with emerging technologies and new experimental designs. With the right improvements, IRT combined with

AI could become a reliable tool for farmers, helping them detect mastitis early, improve cow health, and reduce economic losses.

Key Words: dairy cattle, infrared thermography, artificial intelligence

1502 Validation of a rapid on-farm culture system for group classification of clinical mastitis-causing pathogens. F. G. Guardado Servellon^{*1}, D. L. Renaud¹, B. J. Paredes Osorio², T. J. DeVries³, K. L. Spence¹, and R. Couto Serrenho¹, ¹*Population Medicine, University of Guelph, Guelph, ON, Canada*, ²*Eldale Veterinary Clinic, Elmira, ON, Canada*, ³*Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

The objective of this diagnostic accuracy study was to compare the use of a rapid test tube system (MD; MastDecide, Quidee GmbH, Homberg, Germany) to aerobic milk culture for group classification of mastitis pathogens in dairy cows. A total of 204 milk samples from cows with clinical mastitis (CM) were collected across 60 dairy herds in Ontario, Canada. Clinical mastitis was diagnosed by the presence of abnormal milk or udder changes. Samples were collected by producers and transported to the University of Guelph on the day of collection. Each sample was tested via MD (gram-positive, gram-negative, or “no growth”) and via aerobic milk culture followed by MALDI-TOF (reference method, RM). The MD and RM results were interpreted at 14 and 24 h after incubation, respectively. An additional assessment regarding accuracy of intramammary antibiotic (IMM AB) treatment decisions (gram-positive: to treat; gram-negative and “no growth” samples: not to treat) was performed. Test performance characteristics (overall accuracy, sensitivity [Se], specificity [Sp], positive [PPV] and negative [NPV] predictive values) as well as Cohen’s kappa coefficient (κ) were calculated. The RM results were classified as gram-positive ($n = 107$, 52.5%), gram-negative ($n = 20$, 9.8%), “no growth” ($n = 51$, 25.0%), other pathogens ($n = 21$, 10.3%), and mixed growth (gram-positive and negative; $n = 5$, 2.6%). The RM results for other pathogens and mixed growth were removed for the calculation of test performance characteristics. The Se of the MD test was 58% (95%CI: 47%–67%), 40% (19%–64%), and 61% (50%–72%) for gram-positive, gram-negative, and “no growth,” respectively. The Sp of the MD test was 73% (63%–81%), 84% (78%–89%), and 75% (66%–82%) for gram-positive, gram-negative, and “no growth,” respectively. The PPV and NPV of the MD test were 68% (57%–78%) and 63% (54%–72%) for gram-positive, 22% (10%–39%) and 93% (87%–96%) for gram-negative, and 62% (50%–73%), 74% (65%–82%) for no growth, respectively. The overall test accuracy for Gram group analysis was 57%, with a κ of 0.30. Regarding IMM AB treatment, the MD test yielded a correct decision relative to the RM for 65% of the cases. Using this on-farm test as a primary guide in CM AB treatment decisions should be approached with caution, as the risk of false negatives may potentially compromise cure of CM cases.

Key Words: udder health, selective therapy, dairy cattle

1503 Early genomic predictions for mastitis resistance and somatic cell score association with udder health parameters in Jersey cows. T. J. Almand^{*1}, E. B. Oliveira², H. F. Monteiro¹, D. Bruno³, E. Okello^{1,4}, and F. S. Lima¹, ¹*Department of Population Health and Reproduction, University of California, Davis, Davis, CA*, ²*Zoetis Inc., Kalamazoo, MI*, ³*Cooperative Extension, University of*

Epidemiological studies suggest that Jersey cows generally exhibit a lower incidence of mastitis than Holsteins. However, mastitis remains a significant concern for Jersey cows, indicating that further understanding of the associations of genomic resistance to mastitis (GZMAS) and genomic somatic cell score (GSCS) with mastitis may inform effective management strategies. This study investigated the associations between GZMAS, GSCS, SCC score, and the incidence of clinical and subclinical mastitis in Jersey cows. We hypothesized that cows with higher genomic resistance to mastitis (high GZMAS) and lower predicted somatic cell scores (low GSCS) would experience reduced incidences of clinical and subclinical mastitis, particularly during critical lactation phases. The study included Jersey cows ($n = 2,952$) with at least 200 DIM and available genomic data. The GZMAS and GSCS traits were categorized into terciles (T1 = lowest, T3 = highest), with comparisons focused on 2 extreme groups: low resistance/high SCC (T1T3, $n = 384$) and high resistance/low SCC (T3T1, $n = 413$). Mixed models in JMP (SAS) were used for data analysis using herd and cow ID as random effects. No significant differences in SCC at the previous lactation dry-off were observed (T3T1 = 58.0 ± 20.7 vs. T1T3 = 92.2 ± 23.4 , $P = 0.78$); however, high resistance/low SCC cows had a lower incidence of subclinical mastitis at dry-off (T3T1 = $5.3 \pm 1.6\%$ vs. T1T3 = $14.1 \pm 1.8\%$, $P = 0.003$). At the first test date, these cows exhibited lower SCC (T3T1 = 86.2 ± 22.5 vs. T1T3 = 201.4 ± 25.2 , $P = 0.001$) and reduced subclinical mastitis incidence (T3T1 = $6.4 \pm 1.7\%$ vs. T1T3 = $18.3 \pm 1.9\%$, $P < 0.001$). By 150 DIM, high resistance/low SCC cows had a lower clinical mastitis incidence (T3T1 = $1.3\% \pm 1.3\%$ vs. T1T3 = $10.1\% \pm 1.5\%$, $P = 0.001$), fewer clinical cases per cow (T3T1 = 0.05 ± 0.02 vs. T1T3 = 0.21 ± 0.03 , $P = 0.001$), and fewer subclinical cases per cow (T3T1 = 0.55 ± 0.06 vs. T1T3 = 1.31 ± 0.07 , $P = 0.001$). These findings underscore the potential of genomic selection for mastitis resistance and SCS in guiding targeted mastitis control strategies in Jersey cows.

Key Words: genomics, mastitis, Jerseys

1504 Milk and milk spectra: A rich opportunity for mastitis prediction. D. Lin* and J. A. A. McArt, *Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY.*

Milk Fourier-transform infrared (FTIR) spectra show potential for mastitis prediction, but much remains to be understood about whether they outperform milk yield, SCC, and its predicted milk components (e.g., major components, fatty acids, and metabolites) in a feasible prediction timeframe. In this study, we developed a machine learning framework integrating milk-related features (milk yield and SCC) and milk spectra-related features (4 types of milk major components, 10 types of milk fatty acids, 3 types of metabolites, and milk spectra) for upcoming mastitis prediction, leveraging 2,703 proportional milk samples in the healthy group ($n = 475$; no adverse health events) and 2,819 proportional milk samples in the mastitis group ($n = 441$; diagnosed solely with clinical mastitis), collected from a dairy farm in New York. Each cow was sampled once per day, generating a longitudinal dataset tracking individual cows over time. To capture temporal patterns, we constructed 30 separate predictive models based on subgroups from 30 distinct time periods before the diagnosis date (-30 d to -1 d). To mitigate DIM-related confounding, we applied a data-balancing strategy, ensuring an equal distribution and sample size of healthy and mastitis samples within each subgroup. Additionally, we removed

subgroups with fewer than 10 mastitis samples and excluded samples collected at DIM 0 to reduce physiological variation. From 8 to 6 d before mastitis diagnosis, prediction accuracy increased significantly across all features, with average accuracy improving from 0.42 to 0.69 in regression-based models and from 0.52 to 0.66 in random forest-based models before stabilizing. During this period, the average accuracy of regression-based models using milk spectra improved significantly from 0.37 to 0.81, while random forest-based models showed an increase from 0.52 to 0.75. This improvement was greater compared with other features, such as milk yield (from 0.37/0.54 to 0.66/0.66, regression and random forest, respectively), SCC (from 0.37/0.54 to 0.64/0.64), milk major components (from 0.39/0.50 to 0.65/0.65), milk fatty acids (from 0.55/0.51 to 0.58/0.61), and metabolites (from 0.48/0.39 to 0.58/0.54). Beyond 5 d before diagnosis, while milk spectra remained superior to SCC and its predicted components, its prediction accuracy was no longer consistently higher than that of milk yield (-5 d: 0.81/0.73 vs. 0.78/0.76; -4 d: 0.80/0.70 vs. 0.82/0.80; -3 d: 0.78/0.69 vs. 0.82/0.78; -2 d: 0.77/0.70 vs. 0.82/0.81; -1 d: 0.78/0.71 vs. 0.81/0.80; milk spectra vs milk yield; regression and random forest, respectively). Quantitative analysis confirmed that feature differences between healthy and mastitis groups emerged around 8 d before diagnosis, with milk yield divergence increasing as diagnosis approached. These results highlight the opportunity to use milk-related and milk spectra-related features to predict mastitis earlier, enabling timely interventions and improving farm efficiency.

Key Words: Fourier-transform infrared spectroscopy, milk components, machine learning

1505 Application of machine learning algorithms to predict mastitis events using milk electrical conductivity, rumination time, and water intake parameters. B. Shrestha*, R. Neupane, N. Fagundo, and S. Paudyal, *Department of Animal Science, Texas A&M University, College Station, TX.*

The objective was to evaluate the potential of using milk electrical conductivity (EC), rumination time (RT) and water intake (WI) to develop machine learning based algorithms to predict mastitis events in dairy cattle. This retrospective study was conducted on data collected on an organic dairy farm in central Texas. The final dataset consisted of 925,302 data points, which were collected from 898 dairy cows monitored from 2022 to 2024, with 26,332 event-days labeled as mastitis based on events reported from herd management software (Dairy Comp 305), including 5 d before and after the event as individual events. Daily records of RT (min/d), and WI (L/d) were collected using SmaXtec sensor boluses placed in the rumen of the cows after calving on their first lactation. Electrical conductivity data were retrieved from Afimilk milking system on the dairy. Two different machine learning algorithms: random forest classifier (RF), and AdaBoost classifier (AB) with 5-fold cross validation (80% training, 20% test) were tested using Sklearn Python module to predict the mastitis events using EC, RT, and WI variables individually and in specific combinations using accuracy and precision metrics. The RF model demonstrated better metrics (accuracy %, and precision %) when including all predictor variables (EC, RT, and WI) in the model ($71\% \pm 0.1\%$ and $64\% \pm 0.1\%$ vs. $60\% \pm 0.01\%$ and $51\% \pm 0.2\%$, for RF, AB respectively). Models using the combination of EC and RT together had $71\% \pm 0.1\%$ and $57\% \pm 0.1\%$ versus $67\% \pm 0.01\%$ and $56\% \pm 0.9\%$ values for accuracy and precision for RF and AB, respectively. Models utilizing RT and WI together had accuracy and precision of $70\% \pm 0.1\%$ and $61\% \pm 0.1\%$ versus $60\% \pm 0.01\%$ and $36\% \pm 8.8\%$ for RF and AB, respectively. Models containing EC and WI together had accuracy and precision of $61\% \pm 0.1\%$ and 54%

$\pm 0.3\%$ versus $61\% \pm 0.01\%$ and $52\% \pm 0.6\%$ for RF and AB, respectively. Finally, models utilizing EC only as a predictor had accuracy and precision values $68\% \pm 0.01\%$ and $57\% \pm 0.6\%$ versus $68\% \pm 0.01\%$ and $56\% \pm 0.5\%$ for RF and AB, respectively. These findings suggest that RF model utilizing all 3 variables (EC, RT, and WI) could be used to detect mastitis.

Key Words: accuracy, machine learning, random forest classifier

1506 Multitask contrastive learning for efficient training of small image sets for individual cow recognition across different behavior poses. J. Hooker¹, B. B. de Medeiros¹, C. Saha¹, T. Abdulrahman¹, and A. A. C. Alves^{*1,2}, ¹University of Georgia, Athens, GA, ²Institute for Integrative Precision Agriculture, Athens, GA.

The development of scalable computer vision models for livestock monitoring is often constrained by the need for large, annotated datasets. This study evaluated the effectiveness of multitask contrastive learning (MTCL) for identifying individual Holstein cows from small reference datasets collected under different behaviors and angles. A total of 21 lactating cows were housed in a freestall barn in Winterville, Georgia, where 3 overhead cameras captured RGB images at 10-s intervals. An object detection model (YOLOV7) was trained with 3,120 annotated images to classify 4 behaviors (feeding, lying, drinking, standing). From this set, a random subset was cropped according to labeled bounding boxes and annotated for animal ID, generating a dataset of 557 images (26 ± 8 per animal). We used this dataset to train 2 identification models: (1) a baseline ResNet50-based classifier and (2) an MTCL model, expanding the baseline architecture to a triplet network to learn both cow identification and pairwise similarity. A data augmentation strategy was used for the baseline model to partially mitigate the small image set problem. The MTCL model was trained with 10,000 image pairs sampled from the small dataset to optimize contrastive and cross-entropy losses. To create the testing set, behavior classes and corresponding IDs were manually annotated for an hour (0900–1000 h) across 3 cameras, one day after the training set period. YOLOv7 inferences on the testing set were used to automatically generate cropped images for cow identification models. Behavior detection accuracy in the testing set ranged from 94.75% (standing) to 99.83% (lying down), with sensitivity ranging from 89.00% to 100%. For individual cow identification, MTCL consistently outperformed the baseline model, achieving accuracy between 93.3% (left camera) and 97.4% (right camera). In contrast, the baseline model performed poorly ($<25\%$ accuracy, $F1 < 0.1$) on all behavior poses and angles. The most challenging position for MTCL was standing behavior, with accuracy as low as 89%. Results show that MTCL enhances cow identification across behaviors, supporting robust livestock monitoring with small datasets.

Key Words: animal monitoring, contrastive learning, livestock identification

1507 AI-powered heat stress scoring in individual dairy cow using computer vision and YOLOv11. R. Neupane^{*1}, B. Shrestha¹, S. Muppa², P. Allena², N. Rodriguez³, and J. Velez³, ¹Department of Animal Science, Texas A&M University, College Station, TX, ²Department of Computer Science and Engineering, Texas A&M University, College Station, TX, ³Aurora Organic Dairy, Dublin, TX.

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

The objective was to develop and validate an AI-driven system utilizing computer vision to quantify heat stress in dairy cattle by analyzing

behavioral indicators such as drooling, and open-mouth breathing. A total of 140 cows were selected from a dairy farm in Central Texas and evaluated for panting score by 2 independent observers with Cohen's Kappa value of 0.68 which served as the gold standard. Video footage of study cows was collected using a standard RGB camera (GoPro Hero 9) on 4 d in July 2024. Python pipeline using Os and OpenCV modules were used to extract individual frames from video, and used naive YOLOv11 model for cow detection and following that Segment Anything Model (SAM) for cow image segmentation. The image labeling and annotation was done in RoboFlow, capturing varying lighting conditions and time periods, images preprocessing (resizing and contrast adjustment) and augmentation (flip, and rotation) to generate additional training images ($3\times$). Another object detection YOLOv11 model was trained and employed to detect and segment the muzzle-nose region, followed by the extraction of foam and mucus features. The muzzle-nose detection model achieved a precision of 0.7655, recall of 0.6622, and a mean Average Precision (mAP) of 0.6971 at an IoU threshold of 0.50 (mAP@50), while the foam-mucous detection model had a precision of 0.632, recall of 0.222, and a mAP@50 of 0.224, highlighting the need for further optimization. Heat stress classification was performed using grayscale intensity histograms, and statistical features such as mean and standard deviation of pixel intensities. Based on the value of mean grayscale pixel intensities (AVG), the corresponding heat stress levels were assigned (low [$AVG < 50$], moderate [$50 \leq AVG < 100$], and severe [$AVG \geq 100$]), which achieved an overall classification accuracy of 82%, macro average precision of 56% and macro average recall of 71%. To conclude, we developed and demonstrated an automated heat stress scoring system that reduces reliance on subjective manual observation, provides a scalable solution for real-time cow monitoring. Future work will focus on refining detection models, expanding dataset diversity, and integrating real-time deployment for on-farm applications.

Key Words: artificial intelligence, computer vision, dairy

1508 FT-MIR spectra as management tool for milk processability. I. Alexakis¹, S. Franceschini^{*1}, C. Nickmilder¹, J. Leblois², V. Wolf³, HoliCow Consortium², and H. Soyeurt¹, ¹TERRA Research and Teaching Centre, Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium, ²Elevéo, awé groupe, Ciney, Belgium, ³Conseil Elevage 25-90, Roulans, France.

Milk Fourier transform mid-infrared (FT-MIR) spectra are widely used by the DHIA to select cows based on spectral predictions from milk. However, these spectra remain underused as a management tool for farmers. In the NWE Interreg project HoliCow, 41 million spectra were aggregated from 39 breeds across 6 European countries, enabling the FT-MIR prediction of 26 traits related to milk composition and technological properties. An unsupervised learning approach was then applied to identify patterns in these spectral predictions, focusing on milk technological traits such as laboratory butter yield, cheese yield, and coagulation properties. A specific computational method was developed to manage the large dataset in hierarchical clustering. The dataset was divided into subsets, which were clustered iteratively. At each step, $\sim 95\%$ of the variability was retained from the previous subset and merged into the subsequent subset to process the entire dataset efficiently. The results from each subset were merged, and a final hierarchical clustering was performed on 38,400 centroids. Among the 6 clusters identified, 3 were particularly noteworthy when compared using MIR-based predictions. One cluster exhibited superior aptitude for butter production, characterized by a high butter yield. Another cluster showed the greatest potential for cheese and yogurt production, with improved coagulation properties (i.e., higher curd firmness and coagulation speed), higher cheese yield,

and increased yogurt DM content. The third cluster demonstrated a well-balanced transformation potential, performing well across butter, cheese, and yogurt production. Moreover, multiple algorithms were tested to predict cluster membership based on the 26 predictors. Among these models, random forest achieved the highest overall accuracy in both cross-validation and validation (0.84), effectively capturing the

complexity of the dataset. So, these predictions could be used to guide management on a routine basis and improve the milk quality at individual level to produce dairy products.

Key Words: mid-infrared spectra, pattern detection, management tool

Breeding and Genetics 3: Genetic Selection for Mitigation and Adaptation Traits (Part 1)

1509 Dairy cow resilience: Data-driven detection and quantification of perturbations using daily milk weights. F. L. Guinan^{*1}, R. H. Fourdraine², F. Peñagaricano¹, and K. A. Weigel¹, ¹*University of Wisconsin–Madison, Madison, WI*, ²*Dairy Records Management Systems, Raleigh, NC*.

Resilience is an animal's capacity to recover after a perturbation or maintain specific functions under stress. United States dairy herds group cows into pens based on factors such as parity, lactation stage, and milk production. By coupling daily milk yield data with day-to-day pen location information, we can detect environmental perturbations and measure the responses of individual cows to these stressors over a particular period. Our dataset included 62,580,945 daily milk weights and pen locations of 227,633 cows in parity 1, 2, or 3 from 204 herds representing 30 US states from 2018 to 2024. Individual lactation curves were fitted using polynomial quantile regression with a 0.5 quantile to derive expected lactation curves. Perturbation periods were identified based on residuals between mean expected and mean observed daily milk weights at the pen level. The initial dataset was stratified based on the severity and duration of the perturbation periods, considering 40 combinations of severity levels from $\geq 3\%$ to $\geq 7\%$ and durations ranging from ≥ 3 d to ≥ 14 d. Among the cows that experienced perturbations lasting ≥ 5 d with a severity of $\geq 4\%$, 38% encountered multiple perturbations. Delta milk yield (ΔMY) was measured as the change in a cow's mean daily milk production relative to her mean expected daily milk yield during a perturbation period. The statistical model used to estimate variance components and heritabilities for ΔMY included calving age, DIM, parity, and herd-year-season as fixed effects, along with event and additive genetic and permanent environmental effect as random effects. Sire PTA Pearson correlations with lactation consistency were calculated to assess the relationship between resilience and consistency traits. Estimated heritability of ΔMY during perturbations ranged from 0.01 (0.00) to 0.20 (0.08), while sire PTA Pearson correlations between ΔMY and lactation consistency ranged from -0.31 to -0.06 indicating that more consistent cows have lower milk loss during perturbations. Our findings suggest that cows differ in their response to perturbations at the pen level, and this measure of resilience using daily milk data is heritable.

Key Words: perturbation detection, resilience

1510 Social dominance and feed efficiency: Genetic analysis of latency to first meal after fresh feed delivery and residual feed intake in dairy cows. L. Cavani^{*1}, F. S. Reyes^{1,2}, K. A. Weigel¹, H. M. White¹, and F. Peñagaricano¹, ¹*Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI*, ²*Form-A-Feed Inc., Stewart, MN*.

Social dominance is present in dairy production systems and impacts performance; however, it is difficult to measure. Latency to first meal after feed delivery may serve as a quantifiable phenotype for social dominance, given that dominant cows typically eat first. The goal of this study was to estimate the genetic variability of latency to first meal and evaluate its association with feed efficiency and feeding behavior in dairy cows. Data consisted of 6M bunk visits from 1,770 mid-lactation Holstein cows collected from 2009 to 2024 using an Insentec system (University of Wisconsin–Madison). Latency to first meal was calculated as the time it takes for a cow to access the feed following the first

feed delivery. The statistical model consisted of a repeatability animal model, with lactation and DIM as fixed effects, and cohort, animal, and permanent environment as random effects. Genetic parameters for latency to first meal were estimated using daily records and weekly averages. Heritability and repeatability of latency to first meal using daily records were 0.08 ± 0.01 and 0.22 ± 0.01 , respectively. Heritability and repeatability of latency to first meal using weekly averages were 0.17 ± 0.03 and 0.43 ± 0.01 , respectively. Genetic correlations between weekly averages of latency to first meal and DMI, milk energy, metabolic BW, and residual feed intake were -0.03 ± 0.08 , -0.12 ± 0.08 , 0.10 ± 0.06 , and -0.08 ± 0.10 , respectively. Latency to first meal was negatively correlated with number of visits at the feed bunk per day (-0.65 ± 0.06) and total duration of visits per day (-0.58 ± 0.05). Latency to first meal was positively correlated with duration of each visit (0.39 ± 0.07), intake per visit (0.65 ± 0.06), and feeding rate (0.51 ± 0.07). Overall, cows with longer latencies to eat had fewer but longer bunk visits, greater intake per visit, and ate at a faster rate. Latency to first meal may be a quantifiable phenotype for social dominance, it is both heritable and repeatable, and is not correlated with feed efficiency.

Key Words: behavior heritability milk

1511 The environmental impact of targeted genetic improvement in US beef-on-dairy cattle production. E. Lai^{*} and M. A. Cleveland, *Genus, ABS, DeForest, WI*.

Genetic improvement is increasingly recognized as a promising solution to improve the environmental impact of livestock production, though its contribution has not been fully quantified in commercial systems. Beef-on-dairy (BxD) production is a unique system to examine the effect of genetics because BxD-focused breeding programs are relatively new and, to date, no studies have calculated a BxD environmental footprint. This study presents the first environmental footprint for US BxD production and one of the first life cycle assessments (LCA) accounting for genetic improvement. To quantify the environmental impact of genetic improvement designed to increase BxD production efficiency, a LCA was developed comparing the environmental effect of producing beef from BxD animals sired by industry average or elite genetics. Sire genetic level was determined using a BxD economic selection index: Sires with indices within 0.5 SD of the mean were considered average, and sires with indices at least 1.8 SD above the mean were considered elite. The system boundary included the dairy, rearer, finisher, and harvest phases. The LCA inputs included individual records for feed efficiency and growth from each phase ($n = 3,185$), averaged within each genetic level, and feed composition data. The elite genetic level had a 6.61% lower emission intensity than the average genetic level, 15.38 and 16.47 kg CO₂e/kg carcass weight, respectively, and similarly lower impact across other environmental categories. The lower emission intensity in the elite genetic level was driven by fewer days to finish and faster growth, resulting in lower feed intake, enteric fermentation, and manure production over the animals' lifetime. The finishing phase had the largest impact due to its longer duration and higher feed intake relative to other phases. Therefore, the environmental impact of BxD production will be most responsive to genetic improvement programs that target efficiency gains in the finishing phase. This study demonstrates for the first time that targeted breeding programs can improve the

efficiency of BxD production but also play an important role in reducing the environmental impact of livestock systems.

Key Words: beef-on-dairy, breeding program, sustainability

1512 Investigation of different indicators of resilience in dairy cattle under alternative models. A. Delledonne^{*1,2}, C. Maltecca², F. Tiezzi³, C. Punturiero¹, M. G. Strillacci¹, and A. Bagnato¹, ¹Department of Veterinary Medicine and Animal Science, University of Milan - LA STATALE, Lodi, Italy, ²Department of Animal Sciences, North Carolina State University, Raleigh, NC, ³Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, Florence, Italy.

This study evaluated the impact of lactation curve model selection on assessing resilience in dairy cattle. Fourteen lactation models were compared using data from 1,200 dairy cow lactations recorded via automatic milking systems (AMS) on a Northern Italian farm. Eight established resilience indicators, calculated as deviations between observed and model-predicted (unperturbed) milk yield curves, were analyzed. Results revealed that model choice significantly influenced the ranking of individual cows across resilience indicators. In comparing the Ali and Schaeffer lactation model with the fourth degree polynomial, 76% of the cows ranked within the same top 2% and bottom 2% for the LnVar indicator (logarithm of the variance of the deviations), whereas only 21% were similarly matched when comparing spline regression with the Wilmlink lactation curve. When comparing consistency of rankings LnVar was the most robust indicator across models, while the least robust were those based on negative deviations, such as DPert and WFPert (daily and weighted perturbations), overlapping rankings among models ranging from 1% to 62%. We performed a principal component analysis (PCA) on the models, which indicated high correlation and no specific clustering across models. Loadings within each indicator confirmed consistent patterns across models. But while PCA loadings show that models have similar weights, it is evident that this is not enough to obtain the same rankings. To gain a better understanding of the dynamics of model trait combination we performed a simulation study, which incorporated signal from general physiological variability, random technical error, and resilience related perturbations. Findings of the current study suggest that although model differences exist, underlying patterns of resilience may be consistent across different modeling approaches. The results from this study could be used to refine the definition and measurement of resilience indicators. This study was carried out within the Agritech National Research Center and received funding from the European

Union Next-GenerationEU (PNRR M4 C2 I1.4, CN00000022). This manuscript reflects only the authors views and opinions.

Key Words: resilience, indicators, lactation curve

1513 Are methane sniffer phenotypes useful for genetic ranking of dairy cattle?—From ICAR Feed&Gas working group. R. B. Stephansen^{*1}, A. E. van Breukelen², F. Almasi³, O. González-Recio⁴, E. Teran⁴, V. Milkevych¹, F. Miglior^{5,6}, C. F. Baes^{6,7}, K. A. Bakke⁸, L. Benzoni⁹, R. Finocchiaro⁹, M. Frizzarin¹⁰, N. Krattenmacher¹¹, J. Lassen^{12,1}, J. E. Pryce³, C. Richardson¹³, B. Gredler-Grandl² ¹Aarhus University, Aarhus, Denmark, ²Wageningen University & Research, Wageningen, the Netherlands, ³Agriculture Victoria Research, Bundoora, Victoria, Australia, ⁴Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain, ⁵Lactanet, Guelph, ON, Canada, ⁶University of Guelph, Guelph, ON, Canada, ⁷University of Bern, Bern, Switzerland, ⁸Geno, Hamar, Norway, ⁹Associazione Nazionale Allevatori della Razza Frisone, Bruna e Jersey Italiana, Cremona, Italy, ¹⁰Agroscope, Posieux, Switzerland, ¹¹Kiel University, Kiel, Germany, ¹²Viking Genetics, Randers, Denmark, ¹³AbacusBio, Edinburgh, United Kingdom.

Several countries collect phenotypes for enteric methane (CH₄) emissions, aiming to publish official breeding values in 2025. For genetic evaluations to be accurate, large-scale information on individual CH₄ emissions is crucial. However, gold standard methods such as respiration chambers (RC) can only be used on a small scale. Proxy methods are more suitable for broad use (e.g., sniffers are gaining popularity with >100 installations on commercial farms worldwide). However, sniffers measure CH₄ concentrations rather than absolute emissions, which raises the question whether this impacts the genetic ranking of animals compared with other methods. This literature review and internal data analysis (Table 1) found that phenotypic correlations between sniffers and GreenFeed (GF) data were moderate (0.19–0.30). Using a phenotypic mixed model yielded moderate to strong correlations between sniffers and GF or RC (0.37–0.89), showing the environmental influence. A Dutch study reported high genetic correlations (r_g : 0.71–0.76) between sniffers and GF data, where an across-country analysis showed moderate to high r_g (0.50–0.72) but with high standard errors, indicating mixed models benefited from the relationship matrix for ranking animals. Enhancing processing (e.g., detection of CH₄ signals) of raw sniffer data can increase the heritability, and larger datasets are likely to improve genetic parameter estimation. However, obtaining large datasets from RC is challenging. In conclusion, sniffers enable large

Table 1 (Abstr. 1513). Correlations between sniffer phenotypes and other CH₄ measuring systems in g/d

Item	Country	Hardware	Cows (n)	Correlation (SE)
Phenotypic	Sweden ^R	SN GF	20	0.30 ¹
	The Netherlands ^R		75, 73	0.20, 0.19 ¹
Phenotypic mixed model	Denmark ^R	SN RC	20	0.75 ± 0.20
	England ^R		10	0.75 ± NA
	England ^R	SN GF	2	0.89 ± 0.07
	The Netherlands ^R		2,430	0.39 ± 0.03, 0.37 ± 0.05
Genetic	Netherlands ^R	SN GF	2,430	0.71 ± 0.13, 0.76±0.16
		GF, Canada	2,311	0.59 ± 0.28
	Spain ¹ , SN	SN, Denmark	2,068	0.50 ± 0.25
		SF6, Australia	2,022	0.72 ± 0.10

¹ = Internal data analysis.

^R = Peer reviewed.

¹Transformed R², SN = Sniffer, NA = not available.

scale phenotyping of CH₄ for genetic evaluations, but data processing and modeling are important to ensure accurate ranking.

Key Words: genetic selection, methane, sniffer

1514 ICAR IDF initiative on sensor data for functional traits: Genetics and reference standards for rumination. C. Egger-Danner¹, I. Klaas², L. F. Brito³, J. M. Bewley⁴, V. E. Cabrera⁵, R. H. Fourdraine⁶, M. Haskell⁷, B. Heringstad⁸, M. Hostens⁹, M. Iwersen¹⁰, K. Schodl¹, K. F. Stock¹¹, A. Stygar¹², and E. Vasseur¹³, ¹ZuchtData EDV-Dienstleistungen GmbH, Vienna, Austria, ²DeLaval International AB, Tumba, Sweden, ³Purdue University, West Lafayette, IN, ⁴Holstein Association USA, Brattleboro, VT, ⁵University Wisconsin-Madison, Madison, WI, ⁶Dairy Records Management Systems, NC State University, Raleigh, NC, ⁷SRUC, Edinburgh, United Kingdom, ⁸Norwegian University of Life Sciences, Aas, Norway, ⁹Cornell University, Ithaca, NY, ¹⁰University of Veterinary Medicine, Vienna, Austria, ¹¹IT Solutions for Animal Production (vit), Verden, Germany, ¹²Natural Resources Institute Finland (Luke), Helsinki, Finland, ¹³McGill University, Montreal, QC, Canada.

Although sensor technology offers many possibilities for genetics or quality assurance systems, it is primarily used for on-farm level decision making. Using data from these technologies across farms and along the dairy value chain offers new opportunities for genetic evaluations, welfare assurance, and improved transparency and sustainability in general. The overall aim of the joint ICAR IDF initiative is to develop guidelines to support the adoption of novel technologies and use of sensor data for improving animal health and welfare. During a workshop at the ICAR Conference 2024, the joint initiative discussed motivation and topics of interest for collaboration of different actors, including different stakeholder groups, scientists and manufacturers. A survey among participating manufacturers identified mutual interests and priority areas: establishing a common agreement on definitions and terminology for health conditions and behaviors measured with sensor systems; developing standards to facilitate exchange of data and information across different farms and sensor technologies in accordance and collaboration with other ICAR standards and working groups; make guidelines based on best practices for data collection, handling and analysis for different use (e.g., genetics, health and welfare monitoring); and generating recommendations and protocols for testing the performance of sensor systems. Sensor-based rumination data were selected as the first use case. Preliminary results and recommendations on use of rumination data for genetic evaluation, sensor key performance indicators and preferred reference standards will be presented. Close collaboration between ICAR/IDF with relevant stakeholders and manufacturers of sensor systems enables better understanding of needs and potential of commonly agreed on guidelines, which will improve global adoption of the guidelines to promote use of sensor data in herd management, animal welfare assessment and genetic improvement. Manufacturer representatives: F. Karlson, DeLaval International AB; S. Dagan, J. Heslin, E. Rossi, Allflex Europe SAS; G. Katz, Afimilk Ltd.; M. Moleman, Nedap Livestock; and D. Sieben, Cowmanager B.V.

Key Words: genetic evaluation, herd management, sensor data

1515 Residual heat production: A new trait for dairy cow efficiency? F. Marin^{*2,1}, G. Martinez-Boggio¹, S. J. Kendall¹, M. Carriquiry², H. M. White¹, and F. Peñagaricano¹, ¹University of Wisconsin, Madison, WI, ²Facultad de Agronomía Montevideo, Montevideo, Uruguay.

Feed efficiency is critical in dairy farming as it directly affects both production costs and environmental sustainability. Residual feed intake (RFI) is commonly used to measure feed efficiency; however, it does not fully reflect metabolic efficiency or energy partitioning. As an alternative, residual heat production (RHP) could be used as a measure of energy efficiency. The objective of this study was to evaluate the heritability of RHP and estimate the genetic correlation between RHP and RFI. We individually measured gas emissions (CH₄, CO₂, and O₂, g/d), BW, milk production, milk composition, and DMI over an 8-wk period in 546 mid-lactating Holstein cows housed at 2 University of Wisconsin research farms. Gas emissions were measured using the GreenFeed system and DMI was measured with a roughage intake control system. We calculated heat production (HP, kJ/d) as $HP = 16.18 \times O_2 (L) + 5.02 \times CO_2 (L) - 2.17 \times CH_4 (L)$. Both RFI and RHP were estimated as the difference between the observed and the predicted DMI or HP based on a linear regression including milk energy yield, metabolic BW, change in BW, DIM, and cohort. These 2 efficiency traits are heritable, with heritability estimates equal to 0.29 ± 0.09 and 0.21 ± 0.11 for RFI and RHP, respectively. The phenotypic and genetic correlations between RFI and RHP were 0.42 and 0.56, indicating that these 2 indexes are evaluating efficiency differently and thus, not measuring exactly the same trait. As RHP calculation does not require measuring DMI, it would allow the evaluation of animals in commercial settings. Overall, RHP is a promising trait to evaluate efficiency in dairy cattle.

Key Words: efficiency, heat production, residual

1516 Comparison of residual feed intake and relative breeding values for methane traits. S.-J. Burn^{*1,2}, B. Fuerst-Waltl¹, W. Zolitsch¹, R. E. Jahnel², D. J. Seymour^{3,4}, A. Fleming⁵, C. Egger-Danner⁶, C. F. Baes², and F. Miglior^{2,5}, ¹Institute of Livestock Sciences, Department of Agricultural Sciences, BOKU University, Vienna, Austria, ²Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, ³Trouw Nutrition R&D, Boxmeer, the Netherlands, ⁴Centre for Nutrition Modelling, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, ⁵Lactanet Canada, Guelph, ON, Canada, ⁶ZuchtData EDV-Dienstleistungen GmbH, Vienna, Austria.

Selective breeding is a potential mitigation strategy to reduce greenhouse gas emissions from dairy production. However, recent nutritional research raises concerns that selection for lower methane emissions may negatively affect digestibility. Fecal samples for digestibility analyses might not always be available, so other indicators may be useful. Residual feed intake (RFI) includes unknown factors influencing feed efficiency, such as digestibility. The objective of this study was to use RFI as a proxy for digestibility and to investigate its relationship with relative breeding values (RBV; mean = 100, SD = 5) for 2 methane traits in Canadian Holstein cows. Performance data and RBV were available for 696 primiparous cows. The 2 methane traits evaluated were methane production (MP; g CH₄/d) and methane efficiency (ME: MP genetically independent of milk, fat and protein yield). Cows were divided into 3 classes according to their RBV for both MP and ME: low (RBV <95; n = 69 for MP, n = 68 for ME), medium (RBV 95–105; n = 496 for MP, n = 517 for ME), and high (RBV >105; n = 131 for MP, n = 111 for ME), where a low RBV indicates high emitting and a high RBV indicates low emitting animals. For each cow, an average of weekly RFI was calculated for DIM 120–180. Statistical analyses included an ANOVA to compare group means and Pearson correlation to examine the relationship between RFI and RBV of methane traits. There were no significant differences between the mean RFI of the low (-0.22 ± 0.26), medium (0.08 ± 0.09), and high (-0.38 ± 0.22 ; mean $\pm$ SEM kg DM/d)

RBV ME groups. In contrast, the low RBV MP group had a significantly lower mean RFI (-0.40 ± 0.20) than the medium group (0.13 ± 0.09). The mean RFI of the high RBV MP group (-0.31 ± 0.27) did not differ from the other 2 groups. The overall correlations between the RFI and RBV were not significant for either MP ($P = 0.37$) or ME ($P = 0.87$). These results give first insights that the relationship between RFI and RBV of methane traits in Canadian cows is weak. However, further analyses between actual digestibility and its association with different methane traits are needed.

Key Words: feed efficiency, digestibility, emissions

1517 Genetic evaluation of methane emissions and feed efficiency in Holstein bulls. J. Layton^{*1}, R. Finocchiaro¹, L. Benzoni¹, J. T. van Kaam¹, F. Tiezzi², M. Marusi¹, and J. Cassandro^{1,3}, ¹*Associazione Nazionale Allevatori della Razza Frisone, Bruna e Jersey Italiana (ANAFIBJ), Cremona, Italy*, ²*University of Florence, Department of Agriculture, Food, Environment and Forestry (DAGRI), Firenze, Italy*, ³*University of Padova, Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), Legnaro, Italy*.

Climate change poses a critical global challenge, with the livestock sector contributing significantly to GHG emissions, particularly methane (CH₄) and carbon dioxide (CO₂). Dairy cattle, as a major source of enteric methane, play a pivotal role in the environmental footprint of agriculture. A potential method to reduce CH₄ emissions in dairy cattle is through genetic selection. Based on a paper from 1992, there is indication that measuring and breeding for traits in dairy bulls can have a direct impact on the phenotypic expression of the same trait in their progeny. Since then, little has been done to test the validity of environmental traits measured in Holstein bulls. Hence, the objective was to measure and evaluate the heritability of CH₄ (g/d), feed intake (g/d), and live weight (kg) in growing Holstein bulls between March 2018 and November 2024. A Greenfeed system collected a total of 3,578 valid CH₄ and CO₂ records from 194 bulls. Feed intake was recorded using 5 roughage intake control (RIC) systems, capturing 8,307 feed intake records from 258 bulls, and 2,748 live weight records were collected from 586 bulls in the same time span. Bulls were divided into contemporary and pen groups based on their time of arrival at the genetic center. A single trait ssGBLUP model was developed and tested in BLUPf90 to estimate heritability for CH₄, feed intake, and live weight as 0.29, 0.30, and 0.28, respectively. A third measure, “feed saved while growing,” was obtained by correcting the GEBV for feed intake with live weight. The moderate heritability shown by methane and the growing traits underscores the potential for genetic selection to reduce the environmental impact of dairy cattle.

Key Words: genetic selection, methane reduction

1518 Relationship between milk urea nitrogen and efficiency-related traits in dairy cattle. R. E. Jahnel^{*1}, F. Miglior^{1,2}, C. Machefer¹, S.-J. Burn^{1,3}, F. S. Schenkel¹, and C. F. Baes¹, ¹*Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada*, ²*Lactanet, Guelph, ON, Canada*, ³*Institute of Livestock Sciences, Department of Agricultural Sciences, BOKU University, Vienna, Austria*.

A nitrogen surplus is an indicator for economic losses and increases the overall environmental load of the dairy industry. Milk urea nitrogen in dairy cattle indicates a surplus over the amount of nitrogen needed to maintain their nitrogen balance. Urea is converted from ammonia in the

liver and either recycled into the rumen for microbial protein synthesis or excreted in urine, feces, or milk. Therefore, MUN might be linked to DMI, enteric methane production (MP) and BHB. The objective of this study was to estimate genetic parameters of those traits, including heritabilities and genetic correlations among MUN, DMI, MP, and BHB. A total of 28,220 records of 917 first lactation Canadian Holsteins housed at the Ontario Dairy Research Centre were used in the analyses. Multivariate repeated record models were employed to estimate the variance components using ASReml 4.2. Low to moderate heritabilities were estimated for MUN (0.16 ± 0.08), DMI (0.12 ± 0.08), MP (0.22 ± 0.104), and BHB (0.20 ± 0.099). Genetic correlations between 2 traits ranged between -0.94 ± 0.32 (DMI-BHB) and 0.28 ± 0.07 (DMI-MP). The results indicate that MUN is genetically independent of the other traits, with low genetic correlations between MUN and DMI (0.15 ± 0.09), MUN and MP (-0.05 ± 0.09), and MUN and BHB (0.21 ± 0.39). Phenotypic correlations ranged between -0.11 ± 0.03 (DMI-BHB) and 0.81 ± 0.09 (MUN-DMI). Although no phenotypic relationship could be found between MUN and MP (-0.05 ± 0.08) nor between MUN and BHB (0.01 ± 0.03), the phenotypic expression of MUN is highly associated with DMI. As reflected by the high SE of the estimates, a larger dataset would be needed to confirm these findings. This study is a first step toward a better understanding of possible association of MUN with traits related to feed efficiency, methane emission and energy balance.

Key Words: Canadian Holstein, dry matter intake, methane production

1519 Genetic parameter estimates of diet digestibility in Holsteins and correlations with production. W. Yousaf^{*}, K. J. Harvattine, and C. D. Dechow, *Department of Animal Science, Pennsylvania State University, University Park, PA*.

Our objective was to estimate genetic parameters for diet digestibility in Holsteins and to determine genetic correlations of digestibility with production traits. Fecal samples were collected in the morning and afternoon from 1,064 lactating cows distributed in 6 herds (3 in Pennsylvania, 1 in New York, 1 in Ohio, and 1 in Florida). Samples were collected from each farm twice, and 250 cows had samples collected on both occasions. Diet and fecal samples were dried in a forced-air oven for 48 h at 55°C and composite fecal samples ($n = 1,314$) were made on an equal dry-weight basis. The composite fecal and diet samples were evaluated for NDF and undigestible NDF (uNDF) following a 240-h in vitro digestion. Three digestibility traits were derived including potentially degradable NDF (pdNDF) defined as fecal NDF – fecal uNDF, total-tract NDF digestibility (TTNDFD) and total tract DM digestibility (TTDMD). Yield data included 72,139 test-day records on 4,882 cows and 78,690 SNP genotypes of 5,581 cows for genomic analysis. Digestibility traits were evaluated with a single-step multitrait animal model that considered fixed effects of contemporary group, month of lactation, parity, random additive genetic, random permanent environment, and error. Heritability estimates were 0.15 ± 0.03 , 0.11 ± 0.02 , and 0.08 ± 0.01 for pdNDF, TTNDFD, and TTDMD, respectively, whereas repeatability estimates were 0.25 ± 0.04 , 0.20 ± 0.03 , and 0.16 ± 0.03 , respectively. The additive genetic standard deviation was higher for pdNDF (1.16 ± 0.14) and TTNDFD (1.34 ± 0.13) than TTDMD (0.80 ± 0.06). The genetic correlations of pdNDF with TTNDFD and TTDMD were -0.95 ± 0.02 and -0.21 ± 0.14 . Furthermore, the genetic correlation between TTNDFD and TTDMD was 0.476 ± 0.104 . There was an unfavorable genetic correlation of pdNDF, TTNDFD, and TTDMD with milk yield (0.59 ± 0.10 , -0.74 ± 0.07 , and -0.70 ± 0.04 , respectively). However, the genetic correlations of pdNDF and TTNDFD with fat concentration were favorable (-0.37 ± 0.11 and 0.28 ± 0.09 , respectively). Diet

digestibility is a heritable trait and can be evaluated using commercial farm animals through fecal sampling.

Key Words: NDF, digestibility, heritability

1520 Surface area as a novel digital phenotype for assessing heat stress resilience in dairy cows. A. M. Sallam, E. O'Meara, P. Cardoso, and T. Bresolin*, *University of Illinois at Urbana-Champaign, Urbana, IL.*

Heat stress (HS) is a major global challenge for livestock, with dairy cows being particularly vulnerable due to their high metabolic demands, negatively affecting performance, reproduction, health, and welfare. Identifying cows that maintain baseline performance under HS conditions is important for improving breeding and management strategies. This study explores the potential of surface area (SA) as a digital phenotype for identifying HS-resilient cows. Twenty Holstein dairy cows were subjected to a 9-d HS challenge. Cows were video recorded at 5 time points: 1 wk before HS challenge (T1), 1 d before (T2), 1 d after (T3), 1 wk after (T4), and 2 wk after (T5). An Intel RealSense D435 depth camera, fixed at a consistent height, was used for recording. From

each time point, 5 images per cow were extracted from the videos, and the cow's body was segmented from the background. The SA was measured from each segmented image and averaged within cow and time point to obtain the final SA measurement. SA ranged from 6,422.77 to 9,117.33 cm², with an average of 7,501.09 cm². Surface area significantly decreased from T2 to T3, suggesting that SA can be an indicator trait for HS. At T4 and T5, SA gradually increased toward baseline levels (T1), indicating a prolonged HS effect lasting up to 2 wk after challenge. In addition, SA was used to calculate the recovery rate, maximum deviation, and return to baseline (T1) metrics. Such metrics were then used to cluster cows into 3 groups: resilient, moderately resilient, and sensitive to HS challenge. Resilient cows exhibited minimal deviation, fast recovery, and a rapid return to baseline (T1). Moderately resilient cows showed moderate deviation and recovery. Sensitive cows experienced high deviation, slow recovery, and struggled to return to baseline (T1), reflecting increased susceptibility to the HS challenge. These findings demonstrate the feasibility of using SA as a novel digital phenotype for assessing HS resilience in dairy cows, offering new opportunities for selective breeding and precision livestock management.

Key Words: precision livestock, resilience, selective breeding

Feed Additives to Mitigate Methane Symposium: Recommendations Published in a *Journal of Dairy Science* Special Issue

1521 Recommendations for identification and selection of bioactive compounds to develop antimethanogenic feed additives. Z. Durmic¹, E. Duin², A. Bannink³, A. Belanche⁴, V. Carbone⁵, M. D. Carro⁶, M. Crüsemann⁷, V. Fievez⁸, F. Garcia⁹, A. N. Hristov¹⁰, G. Martinez-Fernandez¹¹, S. Muetzel⁵, E. M. Ungerfeld¹², M. Wang¹³, D. R. Yáñez-Ruiz^{*14}, ¹The University of Western Australia, Crawley, Australia, ²Auburn University, Auburn, AL, ³Wageningen University & Research, Wageningen, the Netherlands, ⁴Universidad de Zaragoza, Zaragoza, Spain, ⁵AgResearch, Palmerston North, New Zealand, ⁶Universidad Politécnica de Madrid, Madrid, Spain, ⁷University of Bonn, Bonn, Germany, ⁸Ghent University, Ghent, Belgium, ⁹Universidad Nacional de Córdoba, Córdoba, Argentina, ¹⁰The Pennsylvania State University, State College, PA, ¹¹CSIRO, Agriculture and Food, Brisbane, QLD, Australia, ¹²Instituto de Investigaciones Agropecuarias, Vilcún, Chile, ¹³Chinese Academy of Sciences, Hunan, China, ¹⁴Estación Experimental del Zaidín, CSIC, Granada, Spain.

This work provides technical recommendations and guidelines for research to identify and screen different compounds to reduce enteric methane (CH₄) emissions before they can be further assessed in vivo. For the initial identification of anti-methanogenic feed additives (AMFA) candidates, 2 approaches can be used: mechanistic (combining computational tools—in silico—and docking studies, using a modeling technique to predict how target enzymes interact with candidate molecules, and then synthesizing the candidate compounds), and empirical (finding published evidence of specific bioactive effects, by searching different repositories, followed by obtaining bioactive material from the identified source). Although both approaches offer potential to identify a large number of compounds, sophisticated equipment and specific expertise are required for computational/modeling work and extracting bioactive compounds from a biological matrix, respectively, in mechanistic and empirical studies. Once AMFA candidates have been identified, the next step is to evaluate experimentally the anti-methanogenic activity and there is an array of in vitro methodologies that can be used for this purpose (enzymatic assays, pure cultures of methanogens batch cultures and continuous or semi-continuous cultures). We will present and discuss the main aspects to consider when using different approaches, including identifying the target enzymes to study, developing the enzyme material for running inhibition kinetics, culturing conditions and selection of archaeal species to study and the processing of the rumen inoculum used for experiments conducted using batch cultures and fermenters. In addition, critical overarching methodological considerations for all in vitro approaches are (1) appropriate experimental design and statistical tests, (2) selection of doses depending on the nature and mode of action of the AMFA, (3) careful preparation of solid or liquid formats for appropriate delivery of the additives, and (4) when and how to measure CH₄ and units to express its production.

Key Words: in silico, docking, in vitro

1522 Recommendations for testing enteric methane-mitigating feed additives in ruminant studies. A. N. Hristov¹, A. Bannink², M. Battelli³, A. Belanche⁴, M. C. Cajarville Sanz⁵, G. Fernandez-Turren^{5,6}, F. Garcia⁷, A. Jonker⁸, D. A. Kenny⁹, V. Lind¹⁰, S. J. Meale¹¹, D. Meo Zilio¹², C. Muñoz¹³, D. Pacheco⁸, N. Peiren¹⁴, M. Ramin¹⁵, L. Rapetti³, A. Schwarm¹⁶, S. Stergiadis¹⁷, K. Theodoridou¹⁸, E. M. Ungerfeld¹⁹, S. van Gastelen², D. R. Yáñez-Ruiz^{*20}, S. M. Waters²¹

and P. Lund²² ¹Department of Animal Science, The Pennsylvania State University, University Park, PA, ²Wageningen Livestock Research, Wageningen University & Research, Wageningen, the Netherlands, ³Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy, University of Milan, Milan, Italy, ⁴Departamento de Producción Animal y Ciencia de los Alimentos, Universidad de Zaragoza, Zaragoza, Spain, ⁵IPAV, Facultad de Veterinaria, Universidad de la Republica, San José, Uruguay, ⁶Instituto Nacional de Investigación Agropecuaria (INIA), Sistema Ganadero Extensivo, Estación Experimental INIA Treinta y Tres, Treinta y Tres, Uruguay, ⁷Universidad Nacional de Córdoba, Facultad de Ciencias Agropecuarias, Córdoba, Argentina, ⁸AgResearch Limited, Grasslands Research Centre, Palmerston North, New Zealand, ⁹Teagasc Animal and Grassland Research and Innovation Centre, Grange, Dunsany Co., Meath, Ireland, ¹⁰Norwegian Institute of Bioeconomy Research, NIBIO, Aas, Norway, ¹¹University of Queensland, Gatton, QLD, Australia, ¹²CREA-Research Center for Animal Production and Aquaculture, Monterotondo, Italy, ¹³Centro Regional de Investigación Remehue, Instituto de Investigaciones Agropecuarias, Osorno, Los Lagos, Chile, ¹⁴Flanders Research Institute for Agriculture, Fisheries and Food, Melle, Belgium, ¹⁵Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences, Umeå, Sweden, ¹⁶Norwegian University of Life Sciences, Ås, Norway, ¹⁷University of Reading, School of Agriculture, Policy and Development, Department of Animal Sciences, New Agriculture Building, Earley Gate, Reading, Berkshire, United Kingdom, ¹⁸Queen's University Belfast, Institute for Global Food Security, Belfast, United Kingdom, ¹⁹Centro Regional de Investigación Carillanca, Instituto de Investigaciones Agropecuarias, Vilcún, La Araucanía, Chile, ²⁰Estación Experimental del Zaidín, CSIC, Granada, Spain, ²¹School of Biological and Chemical Sciences, University of Galway, Galway, Ireland, ²²Department of Animal and Veterinary Sciences, AU Viborg - Research Centre Foulum, Aarhus University, Tjele, Denmark.

There is a need for rigorous and scientifically based testing standards for existing and new enteric methane mitigation technologies, including antimethanogenic feed additives (AMFA). This review provides guidelines for conducting and analyzing data from experiments with ruminants intended to test the antimethanogenic and animal production and physiological effects of AMFA. Recommendations include study design and statistical analysis of the data, interactions with diet composition, associative effect of AMFA with other mitigation strategies, appropriate methods for measuring methane emissions, production, and physiological responses to AMFA, and effects on animal health and product quality. Animal experiments should be planned based on clear hypotheses and experimental designs must be chosen to best answer scientific questions, with pre-experimental power analysis and robust postexperimental statistical analyses being important requisites. Experimental conditions should be representative of the production system of interest, to ensure that results and conclusions are applicable and practical. To explore additivity and synergism, as well as trade-offs, including relevant manure emissions, AMFA may be combined with other methane mitigation strategies and studied in appropriately designed experiments. Methane emissions can be successfully measured, and efficacy of AMFA determined, using respiration chambers, the sulfur hexafluoride method, and the GreenFeed system. For proper assessment of an AMFA, it is critically important that representative animal production data are collected and reported. In addition, evaluating the

effects of AMFA on nutrient digestibility, animal physiology, animal health and reproduction, product quality, and how AMFA interact with nutrient composition of the diet is necessary and should be conducted at various stages of the evaluation process. The authors emphasize that enteric methane mitigation claims should not be made until efficacy of AMFA is confirmed in animal studies designed and conducted considering the guidelines provided in the review.

Key Words: feed additive, enteric methane mitigation, guideline

1523 Feed additives for methane mitigation: Modeling the impact of feed additives on enteric methane emission of ruminants—Approaches and recommendations. J. L. Dijkstra¹, A. Bannink^{*1}, G. Congio², J. L. Ellis³, M. Eugène⁴, F. Garcia⁵, M. Niu⁶, R. E. Vibart⁷, D. R. Yáñez-Ruiz⁸, and E. Kebreab⁹, ¹Wageningen University & Research, Wageningen, the Netherlands, ²Noble Research Institute LLC, Ardmore, OK, ³The University of Guelph, Guelph, Canada, ⁴INRAE - Université Clermont Auvergne, Theix, France, ⁵Universidad Nacional de Córdoba, Córdoba, Argentina, ⁶ETH Zürich, Zürich, Switzerland, ⁷AgResearch, Palmerston North, New Zealand, ⁸CSIC, Granada, Spain, ⁹University of California, Davis, CA.

Over the past decade, there has been significant focus on using anti-methanogenic feed additives (AMFA) to mitigate enteric methane (CH₄) emissions from ruminants. Administered in small quantities, these additives can considerably reduce methanogenesis. Mathematical models are essential for understanding and predicting the quantitative impact of AMFA on enteric CH₄ emissions across diverse diets and production systems. This study provides a comprehensive overview of methodologies for modeling the impact of AMFA on enteric CH₄ emissions in ruminants, concluding with recommendations for modeling approaches to quantify AMFA's impact on CH₄ emissions. Key considerations encompass the type of models employed (i.e., empirical models including meta-analyses, machine learning models, and mechanistic models), alignment of modeling objectives, data availability, modeling synergies and trade-offs associated with using AMFA, and model applications for enhanced understanding, prediction, and integration into higher levels of aggregation. Based on an evaluation of these critical aspects, recommendations are provided for modeling approaches to quantify AMFA's impact on CH₄ emissions and in support of farm-level, national, regional, and global inventories for accounting greenhouse gas emissions in ruminant production systems. These recommendations emphasize that data quality is critical in modeling approaches, with a strong preference for peer-reviewed sources. Careful evaluation of additive dosage, delivery methods, and transient effects is essential in quantitative analyses. The chosen model and modeling approach should be clearly defined and aligned with specific objectives, while exploring the synergy of diverse modeling methodologies, including machine learning, to improve understanding and predictive accuracy of AMFA's impact on CH₄ emissions in ruminants. An integral, quantitative assessment is advised to evaluate AMFA's CH₄ mitigation effects, considering potential synergies or trade-offs with other greenhouse gas sources.

Key Words: feed additive, methane mitigation, modeling

1524 A guideline to uncover the mode of action of antimethanogenic feed additives for ruminants. A. Belanche^{*1}, A. Bannink², J. Dijkstra³, Z. Durmic³, F. Garcia⁴, F. G. Santos⁵, S. Huws⁵, J. Jeyanthan⁶, P. Lund⁷, T. A. McAllister⁸, D. P. Morgavi⁹, S. Muetzel¹⁰, D. W. Pitta¹¹, D. R. Yáñez-Ruiz¹², E. M. Ungerfeld¹³, ¹Universidad de Zaragoza, Zaragoza, Spain, ²Wageningen University, Wageningen, the Netherlands, ³The University of Western Australia, Crawley, Australia,

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We discuss a set of guidelines of research and knowledge required for understanding the mode of action of antimethanogenic feed additives (AMFA). According to their mode of action, AMFA can be classified into 4 categories: (1) lowering H₂ production, (2) inhibiting methanogens, (3) promoting alternative H₂-incorporating pathways, and (4) oxidizing methane. Identifying the exact mode of action of AMFA is complex and costly. Thus, we recommend that in-depth research should only be pursued after the effectiveness and safety of an AMFA has been proven. Key research questions that guide the investigation should cover 5 perspectives: (1) microbiology, identifying the targeted microbes and the potential side effects on nontargeted microbes; (2) cell and molecular biochemistry; identifying the active compounds, the subcellular mechanisms of action on the targeted molecules, and the mechanisms of resistance or adaptation to AMFA; (3) microbial ecology, analyzing the effects of AMFA on the metabolic pathways at the microbial community level, including accumulation of fermentation products and AMFA degradation; (4) animal metabolism, studying effects on feed intake, digestibility, absorption, metabolism, excretion, and accumulation of active compounds or their metabolites to assess safety for animals, consumers, and the environment; and (5) cross cutting, describing the modulatory effects of the diet, type of animal, management, and other factors on the mode of action and effectiveness of AMFA. The research proposed and discussed herein implies multidisciplinary and complementary approaches to fully understand the mode of action of AMFA at different depths. It also addresses existing critical knowledge gaps about the consequences of inhibiting rumen methanogenesis on the microbial ecosystem and host animal, which must be understood for the successful adoption of AMFA to mitigate methane emissions from ruminants.

Key Words: methanogens, mitigation, rumen methanogenesis

1525 Regulations and evidence requirements for the authorization of enteric methane-mitigating feed additives. J. M. Tricarico^{*1}, F. Garcia², A. Bannink³, S. S. Lee⁴, M. A. Miguel⁴, J. R. Newbold⁵, P. K. Rosenstein⁶, M. R. Van der Saag⁷, and D. R. Yáñez-Ruiz⁸, ¹Dairy Management Inc., Rosemont, IL, ²Universidad Nacional de Córdoba, Facultad de Ciencias Agropecuarias, Córdoba, Argentina, ³Wageningen Livestock Research, Wageningen University & Research, Wageningen, the Netherlands, ⁴Sunchon National University, Suncheon, Jeollanam-do, Republic of Korea, ⁵SRUC (Scotland's Rural College), Dumfries, United Kingdom, ⁶Environmental Defense Fund, New York, NY, ⁷Meat & Livestock Australia, North Sydney, NSW, Australia, ⁸Estación Experimental del Zaidín, CSIC, Granada, Spain.

A review of the regulatory and evidence requirements for authorizing feed additives aimed at reducing enteric methane emissions from ruminants was conducted for 7 illustrative jurisdictions. The primary objective was to provide a broad overview and synthesis to help applicants and scientists comply with regulations and understand evidence requirements needed for authorization of antimethanogenic feed additives (AMFA) in Australia, Canada, the European Union, New Zealand, South Korea, the United Kingdom, and the United States. The review also identified differences and similarities in regulations and evidence requirements, which may aid global regulatory harmonization, and

provides recommendations for applicants and scientists. Regulations protect animal health, promote food safety, and prevent unsafe practices and misleading claims. These mandatory regulations cover ingredient safety, manufacturing practices, product labeling, and permissible limits for substances. The intended use, or declared purpose for which the additive is authorized, influences its legal classification or regulatory status, and determines conforming evaluation and approval processes. This includes practical aspects of administration like active ingredients, dosage, mixing, and feeding frequency. Each jurisdiction has unique criteria for legally classifying AMFA, making it challenging to meet all legal classifications with a single set of scientific evidence. However, there is consistency in the need for robust evidence for efficacy, safety, and product quality, despite differences in study requirements. The burden of proof is on the applicant to provide all necessary scientific evidence. Applicants and scientists must consult with authorities before designing research trials. Scientists conduct efficacy and risk assessments that inform regulatory decisions and must follow good scientific practices. Collaboration with regulatory agencies can refine legal classifications and improve harmonization. Educating stakeholders about AMFA's advantages and proper use can encourage correct usage and enhance understanding and transparency.

Key Words: intended use, regulatory status, conditions of use

1526 Feed additives for methane mitigation: How to account for the mitigating potential of antimethanogenic feed additives—Approaches and recommendations. A. del Prado^{*1,2}, R. E. Vibart³, F. M. Bilotto⁴, C. Faverin^{5,6}, F. Garcia⁷, F. L. Henrique⁸, F. F. G. D. Leite⁵, A. M. Mazzetto⁹, B. G. Ridoutt^{10,11}, D. R. Yáñez-Ruiz¹², and A. Bannink¹³, ¹*Basque Centre for Climate Change (BC3), Leioa, Spain*, ²*Ikerbasque—Basque Foundation of Science, Bilbao, Spain*, ³*AgResearch-Grasslands Research Centre, Palmerston North, New Zealand*, ⁴*Cornell University, Ithaca, NY*, ⁵*Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires, Balcarce, Argentina*, ⁶*Universidad Nacional de Mar del Plata, Funes, Mar del Plata, Argentina*, ⁷*Universidad Nacional de Córdoba, Córdoba, Argentina*, ⁸*University of the Republic, Montevideo, Uruguay*, ⁹*AgResearch-Lincoln Research*

Centre, Lincoln, New Zealand, ¹⁰*Commonwealth Scientific and Industrial Research Organisation (CSIRO), Clayton, VIC, Australia*, ¹¹*University of the Free State, Bloemfontein, South Africa*, ¹²*Estación Experimental del Zaidín-CSIC, Granada, Spain*, ¹³*Wageningen University & Research, Wageningen, the Netherlands*.

Recent advances in our understanding of methanogenesis have led to the development of antimethanogenic feed additives (AMFA) that can reduce enteric methane (CH₄) to varying extents. Here we examine current and emerging approaches used for accounting (or quantifying) of enteric CH₄ abatement through the use of AMFA in livestock systems across scales, from the individual animal to the global level. The accounting process for on-farm enteric CH₄ emissions with the use of AMFA starts with the ruminant animal, with estimates obtained from simple empirical emission factors or equations, to complex process-based models. However, the enteric CH₄ abatement from the use of AMFA per se remains to be fully quantified in most accounting systems and scales. The accounting also needs to consider the AMFA delivery method and the accounting of synergies and trade-offs in greenhouse gas emissions at levels before (e.g., emissions from AMFA production and distribution) and after (e.g., effects on feed efficiency) being offered to the animal to fully assess the impact of AMFA use. The choice of methodology and level of complexity to account for AMFA abatement in livestock systems must be tailored to the targeted scale of analysis, the availability of input data to represent contextualized conditions, and the accounting objectives. In the accounting of AMFA abatement, it is critical to consider the implications of efficacy (results from controlled interventions) versus effectiveness (results from real-world conditions). Assumptions on the additivity of AMFA abatement should follow insights from experimental work, and in the future, from models as these continue to improve the simulation of combined AMFA efficacy and effectiveness. Collectively, the accounting of enteric CH₄ abatement by feed additives remains to be fully assessed beyond experimental results to address pragmatism, potential for adoption and societal acceptance.

Key Words: life cycle assessment, emissions trading schemes, emission factors

Reproduction Symposium: Carryover Effects of In Vitro Embryo Production in Dairy Cows—From Embryo Implantation to Next-Generation Performance

1527 The ART of bovine reproduction: In vitro embryo production and its developmental impact. P. Ross*, *STgenetics, Navasota, TX.*

In vitro embryo production (IVP) is transforming bovine breeding by accelerating genetic gain through shortened generational intervals and increased selection intensity. By enabling the large-scale generation of embryos from genetically superior animals at an early age, IVP enhances the efficiency and precision of selection strategies in both dairy and beef systems. However, the preimplantation period—a window of extensive epigenetic remodeling and dynamic gene expression—is highly sensitive to environmental conditions. When this development occurs in vitro, deviations from the in vivo environment can lead to reduced embryo quality, impaired development, lower cryotolerance, compromised pregnancy success, and increased pregnancy loss. Furthermore, IVP-derived embryos have been associated with altered fetal growth patterns, increased birth weight, higher rates of dystocia, and reduced neonatal survival. While the effects of ART on postnatal health and lifetime productivity remain less well documented, emerging evidence suggests potential long-term impacts that warrant further investigation. Importantly, when IVP is used primarily to generate elite sires for genetic dissemination—rather than production animals—the associated developmental risks are less likely to manifest at the farm level. In this context, the benefits of ART in accelerating genetic progress can be realized with minimal impact on commercial herd populations. This presentation explores the balance between genetic advancement and developmental risk in IVP, emphasizing the need for continued refinement of in vitro systems to ensure both reproductive efficiency and the long-term sustainability of genetic improvement programs.

1528 Fertility in lactating dairy cows after timed artificial insemination or embryo transfer with fresh or frozen in vitro-produced embryos. P. Lonergan¹, A. D. Crowe^{1,2}, E. M. Murphy^{1,2}, L. Thompson¹, M. McDonald¹, and S. T. Butler², ¹*University College Dublin, Dublin, Ireland*, ²*Teagasc, Fermoy, Cork, Ireland.*

Reproductive efficiency is crucial for profitable and sustainable dairy production systems. Pregnancy loss, rather than fertilization failure, is a key driver of reproductive failure in cattle. In vitro embryo production (IVP) is now an established technology in the toolbox of assisted reproductive technologies available to farmers and breeding companies for genetic improvement in dairy cow herds. The number of IVP bovine embryos transferred annually has increased year-on-year in the last decade and now surpasses the number derived by traditional superovulation, accounting for ~80% of all bovine embryos produced and transferred globally. In vitro embryo production offers significant advantages over traditional multiple ovulation embryo transfer (MOET) including increased flexibility in sire usage allowing multiple pregnancies from elite dam-sire combinations to be generated, the ability to produce more embryos per unit time per genetically elite female, the ability to use oocytes from prepubertal females (as young as 2 mo of age) to reduce the generation interval (so-called “juvenile in vitro fertilization embryo transfer,” JIVET) and the more efficient use of rare or high-cost semen straws. The increased use of sex-sorted semen to breed replacements and beef semen to improve calf quality in dairy herds will ultimately lead to a reduction in the pool of male dairy calves available as future

potential AI sires. Targeted use of IVP with elite breeding stock will facilitate the production of future generations of elite bulls. Despite these benefits, significant challenges relating to pregnancy loss after embryo transfer, particularly after cryopreservation of IVP embryos, and issues relating to peri- and postnatal health and development of IVP offspring remain to be resolved and hamper the more widespread application of the technology. Improving our understanding of the underlying physiological and molecular mechanisms that regulate early embryo development, embryo-endometrial interactions and lead to successful pregnancy establishment is necessary to understand and elucidate the causes of pregnancy loss and provide a basis for new strategies to improve pregnancy outcomes and reproductive efficiency. Supported by the Irish Department of Agriculture, Food and the Marine Research Stimulus Fund (Grant 2021R665).

Key Words: embryo loss, in vitro fertilization (IVF), assisted reproduction technology (ART)

1529 Impact of in vitro embryo production on the performance of the next generation of dairy cows. S. Lafontaine¹, R. Labrecque², P. Blondin³, R. Côté⁴, and M. A. Sirard⁵, ¹*UQAT, Notre-Dame-du-Nord, QC, Canada*, ²*SEMEX, Saint-Hyacinthe, QC, Canada*, ³*Genome Québec, Montréal, QC, Canada*, ⁴*McGill University, Montréal, QC, Canada*, ⁵*Université Laval, Québec, QC, Canada.*

Assisted reproduction technologies (ART) are key tools in the genomic revolution of the dairy industry. Despite advancements in in vitro fertilization (IVF) and its growing use, concerns remain regarding its long-term effects on animal health and productivity. Research in epigenetics suggests that the artificial environment during early embryonic development may induce subtle metabolic, growth, and fertility alterations that persist throughout life. We explored the hypothesis that epigenetic differences arising from the ovarian and in vitro environment during late folliculogenesis and embryonic culture contribute to long-term phenotypic variations in dairy cows. First, we assessed the epigenetic programming quality of bovine embryos produced in vitro under different culture conditions and donor ages. The DNA methylation analysis of imprinted genes in d 7 and d 12 embryos revealed overall lower methylation levels and greater variability in all 3 in vitro conditions examined. To evaluate the real-world impact of these molecular differences, we conducted a retrospective cohort study (317,888 animals and 601,939 lactations) comparing IVF-derived cows to those conceived via artificial insemination (AI) and multiple ovulation embryo transfer (MOET). While milk production over the first 3 lactations was similar across conception methods when adjusted for genetic merit, IVF animals exhibited slightly reduced fertility, longer gestations, and distinct disease incidence patterns. Although IVF animals had a higher lifetime performance index (LPI), the rate of genetic improvement in their population from 2012 to 2019 was nearly half that of AI-derived cows. We postulate that an epigenetic penalty, consistent with and potentially caused by the molecular differences found in embryos following ART, decreases intergenerational genetic progress and explains the observed phenotypic alterations. Thanks to the quality and quantity of the data available, our work has made it possible to illustrate with unprecedented resolution the causes and long-term impacts of the gametes and early embryos environments. Our findings underscore both the challenges and

progress in minimizing epigenetic dysregulation in elite dairy genetics. Although severe imprinting disorders have been largely mitigated, detectable molecular and physiological traces of ART remain.

Key Words: in vitro fertilization (IVF), phenotypes, epigenetics

Ruminant Nutrition 5: Carbohydrates

1530 Effect of *brachytic2* mutation in corn silage on performance and greenhouse gas emissions of lactating Holsteins fed 2 concentrations of dietary neutral detergent fiber. E. Sarnikaso-glou^{*1}, R. E. Barber¹, D. Hammer², T. Dietz², and M. J. VandeHaar¹, ¹Michigan State University, East Lansing, MI, ²Bayer Crop Science, Chesterfield, MO.

The objective of this study was to evaluate the effect of feeding corn silage (CS) from short stature corn (SHORT), compared with tall corn (TALL), on feed intake, productivity, and greenhouse gas emissions of Holstein cows fed 2 concentrations of dietary NDF. The short stature corn hybrid in this study carried the *brachytic2* (*br2*) mutation gained through natural selective breeding; this mutation results in reduced plant height and improved plant standability. Twenty primiparous and 20 multiparous Holstein cows (76 ± 31 DIM, 46 ± 9 kg milk/d, 658 ± 83 kg BW) were enrolled in a randomized complete block design with 14-d pretreatment period in which all cows were fed a common diet and a 42-d period in which cows were fed 1 of 4 diets. Experimental diets contained SHORT or TALL CS at 2 dietary NDF contents (26% and 31%). All diets were formulated for 18% CP, 3% Fat and fed as TMR. Low-NDF diets had 32% starch and 19% for NDF, and high-NDF diets had 24% starch and 27% for NDF. Data were analyzed using PROC GLIMMIX of SAS 9.4. Orthogonal contrasts were used to determine the effect of CS hybrid, the effect of NDF content, and their respective interaction. Cows fed SHORT CS diets had greater DMI (27.4 vs. 26.4 kg), ECM (44.6 vs. 42.7 kg/d), and protein yield (1.38 vs. 1.33 kg/d) than did cows fed TALL CS diets (all $P = 0.05$). Cows fed high NDF diets had lower DMI (25.8 vs. 28.1 kg), yields of milk (42.6 vs. 45.5 kg/d), and protein (1.28 vs. 1.42 kg/d), but greater ECM/DMI (1.73 vs. 1.64) than cows fed low NDF diets (all $P \leq 0.01$). Lastly, CH4 output (520 vs. 460 g/d; $P = 0.03$), CH4/DMI (20.1 vs. 17 g/kg; $P < 0.01$), and CH4/ECM (11.5 vs. 10.1 g/kg; $P = 0.01$) were greater for cows fed high NDF than low NDF but not altered by corn hybrid. We observed no interactions of hybrid x fiber content, except for DMI (26.7 vs. 24.9 kg; $P = 0.08$), where the decrease in DMI from feeding high NDF was less in diets containing SHORT than TALL. In conclusion, the *br2* CS increased productivity of lactating cows compared with CS for a tall hybrid containing similar NDF and starch content.

Key Words: fiber, performance, short stature corn

1531 Incorporation of sprouted barley in diets of high-yield-ing dairy to partially replace forage on production efficiency and digestibility. U. Moallem^{*1}, L. Lifshitz¹, Y. Shaani^{1,2}, T. Alon^{1,2}, Y. Portnick¹, and H. Leibovich⁴, ¹Department of Ruminant Science, Volcani Institute, Rishon LeZion, Israel, ²Department of Animal Science, the Hebrew University of Jerusalem, Rehovot, Israel, ³Department of Cattle Husbandry, Extension Service, Ministry of Agriculture, Rishon LeZion, Israel, ⁴Israeli Dairy Board, Yehud, Israel.

The sprouted fodder system is a process of growing grains hydroponically and vertically. In a recent study, we included sprouted barley (SB) at 11.5% in the diet and found higher milk, lower milk fat content and yield, and improved digestibility; in this study, we referred to the sprout's NDF as 55% forage-NDF, and we might have overestimated the forage value of the SB; therefore, in the current study, we examined the effects with considering lower NDF-forage values for the SB. Forty-two multiparous mid lactation, Israeli-Holstein cows were divided into 3 groups and fed for 10 wk a diet containing (DM basis):

(1) control (CTL), 36% conventional forage (CF) based on 18.7% corn silage (CS), 14.4% wheat hay (WH), and 2.9% wheat straw (WS); (2) sprouted barley 1 (SB1), 16% SB in which the sprout's NDF was considered as 35% forage-NDF, and 28.7% CF based on 11.4% CS, 8.7% WH, and 8.6% WS; and (3) sprouted barley 2 (SB2), 16% SB in which the sprout's NDF was considered as 45% forage-NDF and 28.1% CF based on 11.4% CS, 8.8% WH, and 7.9% WS. The diets were balanced for starch (~25%), CP (16.5%), NDF (~33%), and forage-NDF content (18%). In the seventh week, 2 rumen samples (2 and 5 h after feeding) were collected for VFA and ammonia, and in the ninth week, 8 fecal samples were collected for digestibility measurements. Production data were analyzed as repeated measurements with the PROC MIXED, and the digestibility data were analyzed with the PROC GLM of SAS. The DMI (31.7, 28.4, and 29.5 kg/d, respectively) and the milk yield (45.3, 41.7, and 42.7 kg/d, respectively) were higher in the CTL than in SB1 and SB2 cows ($P < 0.001$). Milk fat and protein content and yield were not different. The 4% FCM yield was higher in the CTL than in SB1 and SB2 (45.3, 43.1, and 43.8 kg/d, respectively; $P = 0.05$), and the ECM yield was higher in the CTL than in the SB1 cows (45.5 and 43.7 kg/d, respectively, $P = 0.02$). The milk-to-DMI, 4% FCM-to-DM, and ECM-to-DMI ratios were higher in the SB1 and SB2 than in the CTL ($P = 0.001$). Rumen ammonia concentration was higher in the SB1 SB2 than in the CTL ($P < 0.001$), the C2-to-C3 ratio was higher in the CTL than in the SB1 cows ($P = 0.02$), and the total VFA was lower in the SB2 ($P = 0.05$). The apparent total-tract digestibility was higher in the CTL than in the SB1 and SB2 cows ($P = 0.001$) for DM (66.1%, 57.8%, and 60.7%, respectively), OM (68.4%, 60.9%, and 64.0%, respectively), NDF (45.5%, 33.9%, and 37.3%, respectively), and ADF (38.2%, 23.3%, and 27.7%, respectively). In summary, the inclusion of 16% SB, where the sprout's NDF was considered as 35% or 45% forage-NDF, with a relatively high rate of WS in the diet, decreased the intake and yields, but the efficiency was enhanced; however, further research is required to fine-tune the optimal amount of SB in dairy cow's ration.

Key Words: sprouted barley, dairy cow

1532 Effects of concentrate allocation strategy and precision feeding in lactating dairy cows. L. F. Martins^{*1}, S. Issabekova¹, A. T. Richards¹, C. V. Almeida¹, C. J. Eifert¹, D. E. Wasson¹, S. F. Cueva^{1,2}, N. Stepanchenko¹, and A. N. Hristov¹, ¹The Pennsylvania State University, University Park, PA, ²Cargill Animal Nutrition, Shippensburg, PA.

This study investigated the effects of individualized concentrate feeding on lactational performance, ruminal fermentation, and nutrient use efficiency in Holstein cows. Multiparous cows ($n = 48$) averaging ($\pm$ SD) 200 ± 55 DIM, 44.2 ± 7.1 kg/d ECM, with 34.0 ± 3.7 kg/d DMI at the beginning of the study were paired and enrolled in a 12-wk randomized complete block design experiment. Cows were assigned to (1) CON, ad libitum TMR; or (2) PF, precision feeding with ad libitum offering of a partial mixed ration (PMR) formulated to meet NEL and EAA requirements of a cow producing 25 kg/d ECM, and 30% to 60% of concentrate mixtures (CMix) delivered using an automatic feeding system (AFS) to meet individual cow's NEL and EAA requirements. Two CMix were formulated and fed as either high-starch and fatty acids (HSF; 8.2 CP, 53.1 starch, 11.0 FA, % DM) or high-protein (HP; 46 CP, 1.2 starch, 5.5 FA, % DM), based on cow's weekly averaged NEL, Lys, Met, and His balances. Data were analyzed using PROC MIXED of SAS and the statistical models included the fixed effects of treatment, week, treatment

× week interaction, and a covariate measurement. Block and block × treatment were random effects. Precision feeding did not affect NEL balance but decreased ($P \leq 0.10$) excess EAA supply compared with CON. Total DMI (28.0 ± 0.62 kg/d) and ECM (36.0 ± 0.95) kg/d were not affected, but HSF and HP intakes were decreased (-0.4 to -0.8 ± 0.14 kg/d, $P \leq 0.03$) in PF compared with CON. Milk fat content tended to decrease ($-0.17 \pm 0.068\%$, $P = 0.07$) whereas milk true protein and lactose contents were increased (0.06 and $0.08 \pm 0.023\%$, $P = 0.02$) in PF compared with CON. Enteric CH_4 emissions and ruminal fermentation variables were not affected by treatments, except that PF tended to increase ($P = 0.06$) propionate concentration compared with CON. Fecal N tended to decrease ($P = 0.10$), whereas milk N secretion was not affected ($P = 0.22$) by PF compared with CON (27.9 vs. $26.0 \pm 1.27\%$ N intake, respectively). Individualized concentrate feeding via AFS decreased excess EAA supply and improved milk protein and lactose concentrations in mid- to late-lactation dairy cows.

Key Words: methane milk production precision feeding

1533 Effects of replacing dietary starch with sugar on milk yield and composition in high-producing dairy cows. J. I. Norato* and A. L. Lock, *Michigan State University, East Lansing, MI.*

We determined the effect of replacing dietary starch with sugar to evaluate interactions between different carbohydrate sources on milk component yields in high-producing dairy cows. Thirty-six (24 multiparous and 12 primiparous) Holstein cows (153 ± 54 DIM) were randomly assigned to treatment sequences in a replicated 4×4 Latin square design with 4 consecutive 14 d periods with sample and data collection during the last 4 d of each period. Dietary starch and sugar were manipulated by incrementally replacing ground corn with corn gluten feed and sugar beet molasses. Treatment diets (DM basis) were (1) control (CON, 0% molasses), (2) low-sugar (L-SU, 4% molasses), (3) medium-sugar (M-SU, 8% molasses), and (4) high-sugar (H-SU, 12% molasses), maintaining similar forage NDF (20.7% DM), CP (17.3% DM), and total fatty acids (4.25% DM). Formulated dietary starch and sugar contents (% DM) were 32%, 2%, 8%, 24%, and 20%, and 3.51%, 6.44%, 9.36%, and 12.3% for CON, L-SU, M-SU, and H-SU, respectively. The statistical model included the random effect of cow within square and the fixed effects of parity, period, treatment, and their interactions. Preplanned contrasts included the linear, quadratic, and cubic effects of increasing dietary sugar. Results are presented as CON, L-SU, M-SU, and H-SU. There were no treatment-by-parity interactions for the yields of milk and milk components ($P > 0.70$). We observed a linear decrease in the yields of milk (44.5, 44.1, 43.1, and 41.7 kg/d; $P < 0.01$), 3.5% FCM (47.0, 47.2, 46.6, and 45.7 kg/d; $P = 0.05$), ECM (47.3, 47.4, 46.7, and 45.5 kg/d; $P = 0.01$), protein (1.47, 1.45, 1.42, and 1.38 kg/d; $P < 0.01$), and lactose (2.10, 2.07, 2.02, and 1.95 kg/d; $P < 0.01$). However, there was a linear increase in milk fat content (3.88%, 3.96%, 4.03%, and 4.12%; $P = 0.01$) and no effect of treatment on milk fat yield ($P = 0.80$) or body weight ($P = 0.40$). Our results show that under the current dietary conditions tested, replacing starch with sugar linearly increased milk fat content while decreasing the yields of milk, 3.5% FCM, ECM, protein, and lactose, but did not affect milk fat yield or BW.

Key Words: starch, molasses, milk fat

1534 Effects of oscillating dietary starch concentration and cashew nutshell extract supplementation on lactation performance and enteric methane emissions of dairy cows. M. R. Pupo*,

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This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

The objective of this study was to assess the effects of oscillating dietary starch concentration and cashew nutshell extract supplementation on lactation performance, feeding behavior, total-tract digestibility, and enteric methane emissions of dairy cows. Sixty-four multiparous Holstein cows (47 ± 21 DIM) were randomly assigned to 1 of 4 treatments with a 2×2 factorial arrangement for an 8-wk treatment period, after a 1-wk acclimation and 2-wk covariate periods. Treatments were static (NS; 16.9% CP, 28.7% NDF, 25.3% starch) or oscillating (OS; 16.9% CP, 28.8% NDF, 25.7% starch) dietary starch (± 3.1 percentage units) with diet changes at 48-h intervals, and the supplementation without (CON; 16.8% CP, 29.1% NDF, 25.5% starch) or with 6 g/head per day of cashew nutshell extract product (NSE; 17.0% CP, 28.6% NDF, 25.2% starch). Data were analyzed as a completely randomized design with data from the covariate period included as a covariate using a mixed model including starch feeding pattern, feed additive, week, and all their interactions as fixed effects, and cow (treatment) was a random effect. A 3-way interaction was observed ($P = 0.0001$) for DMI, which was about 3 kg/d greater for cows fed NS-NSE than both CON diets during wk 3, 5, 6, and 7 of the treatment period. Cows fed CON tended to have reduced ($P = 0.09$) BW by 2.4 kg/d and daily BW change by 0.09 kg/d than NSE. Minimal effects on performance were observed with NSE and cows fed OS diets. Moreover, greater ($P = 0.02$) meal frequency but reduced ($P < 0.05$) interval between meals, largest interval between meals and eating time were observed for NS than OS diets. The log-variation was greater ($P = 0.01$) for eating time and tended to be greater ($P = 0.08$) for milk yield in OS than NS diets. Digestibilities of DM (69.6% vs. 68.0% DM), OM (70.8% vs. 69.6% OM), and CP (70.2% vs. 68.9% CP) were greater ($P < 0.05$) for NSE than CON, respectively. Additionally, cows fed NSE decreased ($P < 0.05$) methane production (484 vs. 460 g/d) and yield (15.2 vs. 14.2 g $\text{CH}_4/\text{kg DM}$) and tended to decrease ($P = 0.09$) methane intensity. These results suggest that dairy cows may adjust to changes in dietary starch concentration, but the greater variation requires further investigation. In addition, NSE increased digestibility and decreased methane production.

Key Words: cashew nutshell extract (CNSE), feeding behavior, total-tract digestibility

1535 Metagenomic insights into rumen microbial functional responses to high- or low-starch diets in Original Brown Swiss cows. M. Z. Islam^{*1}, J. Lan², H. Zehnle³, Y. Li¹, F. Wahl⁴, E. C. Slack², M. Schoelmerich³, R. Zenobi⁵, S. Giannoukos⁵, S. E. Räisänen^{1,6}, and M. Niu¹, ¹Animal Nutrition, Department of Environmental Systems Science, ETH Zürich, Zürich, Switzerland, ²Department of Health Sciences and Technology, ETH Zürich, Zürich, Switzerland, ³Environmental Microbiology, Department of Environmental Systems Science, ETH Zürich, Zürich, Switzerland, ⁴Food Microbial Systems Research Division, Agroscope, Bern, Switzerland, ⁵Department of Chemistry and Applied Biosciences, ETH Zürich, Zürich, Switzerland, ⁶Department of Animal and Veterinary Sciences, Aarhus University, Tjele, Denmark.

This short-term study investigated the effects of dietary starch level on rumen microbial metabolism using a metagenomic approach as part of a multi-omics integration project. A switchback design with three 9-d periods (7-d adaptation, 2-d sampling) was used, with 4 rumen-cannulated Original Brown Swiss cows assigned to low-starch (LS,

6.31% DM) and high-starch (HS, 16.2% DM) diets in an ABA/BAB sequence. Rumen content (solid+fluid) was collected 2-h after feeding for metagenomic analysis. Taxonomic profiles were estimated using SingleM from metagenomic raw reads, while bacterial and archaeal abundances were assessed using MetaCoder (Wilcoxon test, adjusted $P \leq 0.10$). Functional profiling was performed by predicting open reading frames from assembly scaffolds (>1,000 base pairs) using Prodigal, followed by Hidden Markov Model searches against the KOfam database. To compare the diet effects (LS vs. HS), KEGG orthologs were grouped into 11 broad functional categories. Feature-level differential abundance was analyzed using MetagenomeSeq (adjusted $P \leq 0.10$, log2 fold-change with cumulative-sum scaling). Pathway enrichment analysis was conducted against the KEGG database. The taxonomic analysis revealed the dominance ($P < 0.05$) of *Bacteroidia* in LS and

Clostridia in HS diet. Functional diversity profiling showed that carbohydrate and glycan biosynthesis pathways were relatively abundant in both diets. Differential analysis of KEGG orthologs did not identify discriminative features. Enrichment network analysis revealed that carbohydrate metabolic pathways were enriched ($P < 0.05$) across both diets, particularly in glycolysis, pyruvate, galactose, starch and sucrose metabolism. Overall, the taxonomic profile indicated that fiber-digesting *Bacteroidia* and *Clostridia* were more abundant in LS and HS diets, respectively, although the diet did not alter the feature-level functional profile. Although metagenomics revealed minimal differences between diets in this short-term study, the ongoing analyses may uncover deeper insights into active microbial metabolism through metatranscriptomics.

Key Words: metagenomics, rumen microbiome, rumen function

ADSA Dairy Foods Division International Partnership Program (IPP)

Symposium: The Netherlands Food Valley—Protein and Lipid Functionalities for Future Dairy Products

1536 Tailoring digestion of dairy proteins through processing to obtain specific structures. K. A. Hettinga*, *Wageningen University, Wageningen, the Netherlands.*

Milk proteins, as one of the major protein sources in the Western diet, are nutritionally important, but also have numerous other physiological effects. When consuming milk proteins, these are usually present in a specific product structure that may modulate the physiological effects. Processing of milk changes the physicochemical properties of milk proteins and the properties of the resulting dairy product structure. These changes will subsequently have consequences for digestibility. Postdigestion, this may lead to different digestion products being present in the intestine, having both nutritional and immunological consequences for the consumer. The impact of milk processing on protein functionality depends on both structure and composition of the dairy matrix as interactions among proteins, as well as between proteins and other milk components, will occur. For example, the presence of β -LG during heating changes aggregation among proteins, but also the presence of minerals and lipids modulates process-induced changes to milk proteins. These process-induced changes are known to impact the formation of dairy structures (e.g., yogurt, cheese), and may also impact dairy structures during digestion (e.g., gastric clot formation). The physiological consequences of these process-induced effects on dairy product structure have been studied using both in vivo and in vitro approaches, with the integration of data from both types of studies giving novel insights into the health impact of processing on dairy products. In this presentation, I will therefore discuss the effect of industrial processes on milk protein properties and the effect of dairy structures, showing how this will impact digestion of dairy products and the nutritional and immunological consequences thereof. This knowledge can be used for tailoring digestion of dairy products by using specific processes.

Key Words: milk protein, structure, digestion

1537 The genetic background of bovine and goat milk composition in relation to functional properties. H. Bovenhuis*, R. Gonzalez-Prendes^{1,3}, Y. Liu¹, E. Bijl², and R. P. M. A. Crooijmans¹, ¹*Wageningen University & Research, Animal Breeding and Genomics, Wageningen, the Netherlands*, ²*Wageningen University & Research, Food Quality and Design Group, Wageningen, the Netherlands*, ³*Ausnutria BV, Zwolle, the Netherlands*.

Milk is a unique product rich in essential nutrients, including vitamins, minerals, amino acids, and fatty acids. Significant differences exist among individual cows and goats in milk composition, partly due to genetic factors. Historically, these genetic differences have been utilized to tailor milk composition for specific dairy products. For instance, selection for increased fat content was driven by demand for butter, while selection for protein content was influenced by cheese production. Over time, this selective breeding resulted in distinct breeds and, in some cases, differences in breeding goals within the same breed across different countries. Extensive research has established that milk protein variants influence manufacturing properties. Variants of kappa-casein have been linked to milk's renneting time, while β -LG variants affect the fouling rate of heating equipment. The availability of whole genome

sequence data presents exciting opportunities for identifying additional milk protein variants. Beyond these qualitative genetic differences in milk protein variants, more recent studies have revealed substantial quantitative genetic variation in milk protein composition. Similarly, genetic factors play a significant role in variations in milk fatty acid composition, mineral content, and oligosaccharides. Several genes and genomic regions with a major influence on detailed milk composition have been identified. Despite its potential, this genetic variation remains largely untapped, while insights at the DNA level offer interesting possibilities for the production of specialized dairy products.

Key Words: milk, genetic, composition

1538 Goat milk caseins: Insights into their compositional variability and technofunctionality compared with cow milk caseins. S. Breunig*, H. Bovenhuis³, R. P. M. A. Crooijmans³, P. Keijzer², K. A. Hettinga¹, and E. Bijl¹, ¹*Food Quality and Design Group, Wageningen University & Research, Wageningen, the Netherlands*, ²*Ausnutria B.V., Zwolle, the Netherlands*, ³*Animal Breeding and Genomics, Wageningen University & Research, Wageningen, the Netherlands*.

Goat milk products often exhibit different properties than cow milk products, for example during gelation. As casein micelles are key structural elements for many dairy products, studying goat milk casein micelles and their techno-functional properties is essential to improve our understanding of goat milk and products thereof. We studied the compositional variation of goat caseins in individual milk samples with regard to casein composition, casein micelle size, protein content, and salt composition, which are interconnected. The occurrence of certain genetic goat casein variants thereby impacts the overall casein composition and casein micelle size. The interaction between these different parameters is complex, especially because goat milk casein is highly polymorphic. Furthermore, the interplay between these parameters is not always similar to cow casein micelles and is therefore important to investigate. Despite differences in casein composition, micelle size, and hydration between goat and cow caseins the internal structure of casein micelles was found to be similar using small-angle neutron scattering (SANS). Gelation properties such as gel strength and coagulation time of raw goat and cow milk also only differ slightly. The gelation properties of heated cow and goat milk, however, are changed significantly due to heat-induced aggregation of whey proteins and caseins. However, these heat-induced changes are more severe for cow milk gels. We show that this difference is due to greater heat-induced changes on cow than goat casein micelles. We furthermore investigated whether this is mainly influenced by differences in casein or whey fractions in both types of milk. Differences in the gel structure formed from raw and heated goat and cow milk were also observed using spin-echo SANS. This approach showed that heat-induced differences in gel structure are mainly due to changes in the compactness of casein aggregates formed during gelation. In this presentation, we will thus cover novel insights into goat caseins and their variation, their similarities to cow caseins, as well as some of the key differences that appear to be relevant for techno-functional properties.

Key Words: goat milk, casein micelle, milk gelation

1539 Dairy innovations and their relevance for immune health. B. van Esch^{1,2}, T. Baars¹, M. Diks¹, P. Dekker^{3,4}, K. A. Hetinga³, R. Kort⁵, and J. Garssen^{*1,2}, ¹*Faculty of Science, UIPS, Division of Pharmacology, Utrecht University, Utrecht, the Netherlands*, ²*Danone Nutricia Research, Utrecht, the Netherlands*, ³*Food Quality and Design Group, Wageningen University & Research, Wageningen, the Netherlands*, ⁴*Laboratory of Biochemistry, Wageningen University & Research, Wageningen, the Netherlands*, ⁵*Amsterdam Institute for Life and Environment, Vrije Universiteit Amsterdam, Amsterdam, the Netherlands*.

Epidemiological studies have shown an inverse relation between raw cows' milk consumption and the development of allergies. This protective effect seemed to be abolished by milk processing. In recent years we confirmed the epidemiological findings on asthma and food allergy by showing causality. Current research is aiming at mild-thermal and non-thermal processing techniques and the relevance for immune health. Pathogen-controlled raw milk was collected from a biodynamic dairy farm. For kefir, raw milk (Raw Milk Company, the Netherlands) was fermented with a defined freeze-dried starter culture. Allergy modulating effect of raw milk was assessed in murine models for allergic asthma and food allergy and a proof-of-concept provocation trial in infants allergic to cow milk. Kefirs made from either raw or heated milk were investigated for bacterial and fungal microbiota composition, and the allergy modulating effect. Raw milk reduced allergic symptoms in models for asthma and food allergy. Effects were preserved after skimming but abolished after pasteurization. Bioactive proteins in the whey fraction are partly responsible for the effects. The allergy protective effect of raw milk was lost around 65°C. We demonstrated that raw unprocessed cow milk has a lower allergenic potential than processed milk. The latter was confirmed in allergic children who tolerated raw cow milk up to 50 mL without showing any allergic symptoms. In kefir made from either raw or heated milk, we identified variants identical to those in the starter culture. In raw milk kefir, specific variants of *Lactococcus lactis* and the yeasts *Pichia* and *Galactomyces* could be identified. The number and intensity of peptides drastically increased after fermentation. Heating negatively affected the diversity of the peptide composition in kefir. Raw milk kefir suppressed the acute allergic skin response in a murine food allergy model. These effects coincided with higher percentages of activated Th1 cells and IFN γ production. Raw and low temperatures processed cow milk reduced allergic symptoms in experimental models for food allergy and allergic asthma pointing toward a generic immune effect of unprocessed cow milk. Nonthermal processing techniques such as fermentation are promising tools for producing a safe dairy product with preserved health benefits.

Key Words: immune health, dairy, processing, raw milk

1540 Functional properties and sensory perception of dairy-based products. E. Scholten* and G. Sala, *Wageningen University, Wageningen, the Netherlands*.

Controlling the sensory experience of food products is essential to increase food liking. Different sensory attributes create a specific mouthfeel, which is governed by structure that the different ingredients create through a variety of interactions. The structure provides certain macroscopic rheological (mechanical) properties and lubrication aspects, important for many different sensory attributes, such as hardness, springiness, adhesives, creaminess, and so on. Understanding the link between the specific organization of the structural elements at smaller length scales and the textural properties on a macroscopic scale is essential to influence sensory perception. In this presentation, we will discuss the link between structure and texture of different dairy

products. For example, in the case of cheese, we will discuss the role of different enzymes on the textural evolution related to protein hydrolysis and the creation of the casein network and accompanying interactions. The specific structural characteristics drive a variety of different sensory attributes, both during the first bite and during oral processing. In the case of heated gels, we will discuss how specific ingredients and additives, such as fat and salt alter the structure of the gels at different pH values and conditions, and how this is linked to different textural characteristics, such as the fracture properties and melting behavior, and how these are related to sensory perception. In ice cream, we show how dairy fat controls the complex structure in terms of the overrun, the serum phase properties, and the ice crystal network, and how this is linked to different quality attributes in ice cream, such as mechanical and melting properties. These properties depend on both the formation of partially coalesced fat globules at the air-water interface and the formation of a continuous fat network in the lamellae present between the air cells. Such knowledge provides an understanding of structure-texture relationships, which can then also be used for ingredient flexibility. We will briefly discuss how this knowledge can also be used for product reformulation, and the design of sugar and fat-reduced ice cream or dairy-free cheese.

Key Words: cheese, ice cream, milk fat

1541 Effects of prepartum dam characteristics on colostrum and heifer calf development. Y. P. Wang^{*1}, E. Saccenti², A. Lammers¹, J. Arts¹, R. M. Bruckmaier³, E. E. A. Burgers^{1,4}, R. M. A. Goselink⁴, J. J. Gross³, M. Reichelt¹, B. Kemp¹, and A. T. M. van Kneegsel¹, ¹*Adaptation Physiology Group, Wageningen University & Research, Wageningen, the Netherlands*, ²*Laboratory of Systems and Synthetic Biology, Wageningen University & Research, Wageningen, the Netherlands*, ³*Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland*, ⁴*Wageningen Livestock Research, Wageningen University & Research, Wageningen, the Netherlands*.

The objective of this study was to evaluate the relationships between dairy dams and their heifer calves by first analyzing the effects of prepartum metabolic and milk characteristics of dams on their heifer calves, and second, analyzing the role of colostrum yield and composition in the dam-calf relationships. Holstein Friesian dairy dams (n = 62) were monitored from 4 wk before conception until calving. Heifer calves from those dams were monitored from birth until the first 100 DIM of their first lactation. Dam variables were classified according to the 4 life stages: preconception and the 3 trimesters of gestation. Calf variables were classified according to the 3 life stages: weaning, rearing, and first 100 DIM. Dam and heifer variables were analyzed one-by-one in a regression model per life stage. Higher milk lactose in dams during the preconception period and the first 2 trimesters of gestation was related to higher BW in calves during rearing and first 100 DIM. Higher BCS in dams during the third trimester of gestation was related to lower concentrations of lactoferrin, IGF-1, and transforming growth factor- β 2 in colostrum, which were further related to lower plasma nonesterified fatty acid concentration in calves during weaning. Greater prepartum dry matter intake and energy balance in dams were related to higher natural antibody (NAb) levels in dam colostrum which were further linked to higher NAb levels in calf plasma during weaning and rearing. In conclusion, the greatest percentage of relationships occurred when dams and heifers were both lactating, compared with weaning and rearing life stages in calves. Colostrum composition played a role in the relationship between dam milk yield and energy status with calf BW and immune variables.

Key Words: epigenesis, maternal effects, dam-calf relationships

1542 Reassembled casein micelles as a model system to study casein micelles. Z. Fan*, E. Bijl, and K. A. Hettinga, *Wageningen University & Research, Wageningen, the Netherlands*.

Casein micelles are complex colloidal particles with essential functional and nutritional roles in milk and dairy products. The casein micelle structure is important for dairy product manufacturing, as the gelation of casein micelles is a key step in producing dairy products such as cheese and yogurt. Therefore, understanding the structure and behavior of casein micelles is critical. However, casein micelles exhibit natural variation in casein composition, post-translational modifications, mineral content, and size, making them a difficult system to study. For example, although the casein composition of casein micelles has been linked to their rennet coagulation properties, the precise relationships and underlying mechanisms remain inconclusive. To address these challenges, we need a model system that is easier to control and study, such as reassembled casein micelles (RCM). The RCM are casein micelles assembled in vitro by slowly mixing purified caseins and milk salts under controlled conditions (typically 37°C and pH 6.7). By making reassembled casein micelle, we can isolate a single variable and study how it affects casein micelle structure and behavior. Additionally, by modifying the structure of RCM, we can connect their structural variations to functional properties, such as gelation. This presentation will demonstrate how RCM have been used to investigate the effects of casein composition and modifications on casein micelle formation and structure, as well as the connection to gelation.

Key Words: reassembled casein micelles, casein micelle structure, casein micelle gelation

1543 Recombinant milk proteins for the development of high-quality dairy alternatives. E. Bijl*, A. De Groot, Z. Fan, R. Bouma, A. E. Thiel, L. M. C. Sagis, and K. A. Hettinga, *Wageningen University, Wageningen, the Netherlands*.

The consumer demand for dairy alternatives has been rapidly growing in the past decades. Concerns about sustainability and animal welfare of

traditional milk production are 2 important motivations for an increasing percentage of consumers to replace animal-based proteins with plant-based protein sources. Unfortunately, current plant-based analogs of dairy foods on the market have sensory and techno-functional shortcomings. An emerging alternative is the use of genetic engineering to produce dairy proteins during the process called "precision fermentation." For this process, recombinant DNA technology is used to insert protein genes into fast-growing host organisms such as bacteria, yeast, and fungi. This way, recombinant dairy proteins, such as β -LG, caseins, or lactoferrin, can be produced. A significant advantage of recombinant-produced proteins is that they have, in contrast to plant-based proteins, similar or even an improved composition and structure compared with the original animal-based dairy proteins. Using these ingredients therefore allows for a fine-tuned modulation of dairy product quality. We are currently working on research projects involving these recombinant proteins to develop microbial-based alternatives for dairy ingredients and products with a specific focus on the role of caseins. We used highly purified casein fractions obtained from bovine and microbial-based sources. Foaming and emulsifier properties were investigated by determining the properties of the air-water and oil-water interfaces using oscillating drop tensiometry. In addition, the assembly of these caseins into casein micelles increases the application for dairy products, such as cheese and yogurt, as the coagulation properties of casein micelles shape the structure of these products. We therefore determined how renneting, acid gelation and gastric clotting of casein micelles assembled in vitro differ from native casein micelles. By understanding structure-formation-stability-function relationships between caseins and reassembled casein micelles, we can tailor these to optimize functionality for high-quality alternatives for dairy ingredients and products. Simultaneously, this increased understanding allows us to translate these insights back to regular dairy products.

Key Words: caseins, precision fermentation, gelation

Animal Health 5: Transition Cows

1544 Sustainability impacts associated with clinical cure failure of metritis in US dairy herds. A. Martelo-Pereira^{*1}, M. I. Marcondes^{1,2}, and C. C. Figueiredo¹, ¹*Washington State University, Pullman, WA*, ²*The W.H. Miner Agricultural Research Institute, Chazy, NY*

This study aimed to assess the economic and environmental impact associated with clinical cure failure of metritis in dairy herds. Clinical cure failure of metritis, characterized by the persistence of watery, reddish-brown, and fetid vaginal discharge after antimicrobial treatment, affects 20% of metritic cows. Considering the incidence of metritis at 25% in US dairy herds and the occurrence of clinical cure failure (20%), a Markov Chain model that engineered 10 yr of performance of Holstein dairy cows was used. The model was defined by the cow's physiological state and transition probabilities, where the cow's state was categorized according to the cow's category (e.g., heifer, lactating, or nonlactating) and the transition probability. The model incorporated metritis incidence and used a Solver to adjust the voluntary culling rate for cows with 3 or more lactations (ranging from 0% to 40%) and the number of heifers sold at birth, aiming to stabilize the herd and maximize annual profit. The model was constrained to a minimum of 900 and a maximum of 1,105 milking cows. All cows with metritis were treated with ceftiofur crystalline-free acid, costing \$108.00/cow. Data from the tenth year was computed to compare the profit between 3 different dairy herd scenarios: Base: dairy herd with 0% incidence metritis; Cured: dairy herd with 25% incidence metritis (100% cure risk); Noncured: dairy herd with 25% incidence metritis (0% cure risk; Table 1). Noncured herds produced less milk and sold more animals, leading to reduced annual profit and higher costs per metritis case than cured herds. Higher phosphorus and methane emissions were observed in noncured herds than in cured herds. In conclusion, this study highlights that the clinical cure failure of metritis significantly impacts the economic and environmental sustainability of dairy operations.

Key Words: profitability, methane, uterus

1545 Anti-inflammatory treatment at dry-off: Effects on metabolic status in high-priority cow groups—Part II. R. Sorto^{*1}, R. Landrum¹, M. Molins², L. Periconi¹, M. Marinelli¹, K. J. Harvatine², T. Ott², E. Hovingh¹, and A. A. Barragan¹, ¹*Department of Veterinary and Biomedical Sciences, Penn State University, State College, PA*, ²*Department of Animal Sciences, Penn State University, State College, PA*

Table 1 (Abstr. 1544).

Item	Base	Cured	Noncured
All animals, n	1,723	1,741	1,862
Milking cows, n	986	962	937
Milk yield/cow, L/d	41	39	35
Milk revenue, \$	6,877,869	6,450,512	5,638,412
Animal sales revenue, \$	646,172	671,203	861,599
Profit, \$/yr	1,086	883	545
Cost per metritis case, \$	0	842	2,276
Total P, g/L	1.23	1.29	1.49
Total CH ₄ , g/L	14.21	14.65	16.10

The objective of this study was to assess the effects of acetylsalicylic acid administration at dry-off on the metabolic status (i.e., nonesterified fatty acid concentration [NEFA], BHB, and BCS) during the transition period in Holstein dairy cows. On the day of dry-off, cows were blocked by parity (primiparous [PRIM]; multiparous [MULT]) and randomly allocated to one of 2 treatments: ASA (54) = receive an oral treatment with 4 acetylsalicylic acid boluses (125g/cow); or PLC (53) = receive an oral treatment with 4 gelatin capsules filled with water. The BCS was measured at dry-off to group cows in BCS categories (BCSDRYCAT; optimal [OPT] = 2.75–3.5 pts.; over-conditioned [OVERC] = 3.75–5 pts.). Milk samples were collected at dry-off in a randomly selected subgroup of cows (ASA = 28, PLC = 23) to measure SCC, and group cows into SCC categories (SCCDRYCAT; LOWSCC <200,000; [n = 31], HIGHSCC ≥200,000; [n = 20]). Blood samples were collected for assessment of NEFA and BHB, and BCS was assessed, weekly until 21 DIM. The data were analyzed using the UNIVARIATE and MIXED procedures of SAS as a randomized complete block design. A tendency for an ASA × BCSDRYCAT interaction was observed, where OVERC cows treated with ASA tended ($P = 0.07$) to have a lower prepartum NEFA concentration compared with PLC OVERC cows (ASA-OVERC = 179.68 mEq/L, 95%CI: 165.4–195.2; PLC-OVERC = 196.29 mEq/L, 95% CI: 180.2–213.8). In the SCC cohort, there was a tendency for interaction between SCCDRYCAT, treatment, and day, where HIGHSCC cows treated with ASA had lower NEFA concentration at 35 ± 3 and 21 ± 3 d before caving compared with PLC HIGHSCC cows. A treatment-by-day interaction was found on postpartum BHB concentration ($P = 0.05$). Cows treated with ASA had a higher concentration of BHB at 7 ± 3 DIM, while PLC cows had a higher concentration of BHB at 21 ± 3 DIM. In addition, cows treated with ASA have higher BCS in the first week after calving compared with PLC cows ($P = 0.04$). These results suggest that ASA treatment at dry-off may improve concentrations of metabolic parameters in specific group of cows, such as OVERC and HIGHSCC, often considered high-priority animals.

Key Words: dry-off, anti-inflammatory treatment, metabolic status

1546 Effects of feeding rumen-protected choline 21 days prepartum to 100 days postpartum on lactation, health, reproduction, and culling of Holstein dairy cows. M. J. Wollentarski^{*1}, T. C. Marques¹, D. Melo¹, W. M. Coelho Jr.¹, H. F. Monteiro¹, R. Profeta¹, M. C. G. M. Silva¹, A. Ueda¹, E. Raffrenato², D. K. Dubey³, and F. S. Lima¹, ¹*Department of Population Health and Reproduction, University of California, Davis, Davis, CA*, ²*Kemin Industries Inc., Des Moines, IA*, ³*Kemin Europa NV, Herentals, Belgium*

Our objective was to evaluate whether extending rumen-protected choline (RPC) supplementation from 21 d prepartum to 100 d postpartum improves lactation, health, culling, and reproductive performance in a large commercial dairy herd. Holstein cows were blocked by parity and randomly assigned to the control (CON; n = 389) or RPC (n = 385) group. Cows were fed as pen/cohort (n = 28), the experimental unit, with individual cows as observational units. The RPC group received 15 g/d prepartum (–21 to 0) and 30 g/d postpartum (0 to 100 d) of RPC (Choli-GEM, Kemin Industries Inc.). Control cows only received RPC from 0 to 21 DIM. Data were analyzed using mixed models, Cox proportional hazard models, and logistic regression in JMP. Health outcomes did not differ significantly between groups for metritis (CON = 4.9% ± 2.8% vs. RPC = 3.1% ± 2.7%; $P = 0.22$) or mastitis (CON = 38.0% ± 7.5% vs. RPC = 37.4% ± 7.0%; $P = 0.88$). However, RPC tended to reduce

stillbirth incidence (CON = 8.2% ± 3.5% vs. RPC = 4.2% ± 3.4%; $P = 0.10$) and significantly lowered culling rates (CON = 36.5% ± 6.2% vs. RPC = 27.1% ± 6.1%; $P < 0.001$). Interestingly, RPC-fed cows had a delayed first estrus (CON = 34.0 ± 3.8 d vs. RPC = 41.3 ± 3.7 d; $P < 0.01$) but showed no differences in time to first artificial insemination (CON = 76.7 ± 2.2 d vs. RPC = 77.2 ± 2.1 d; $P = 0.85$) or first-service pregnancy per AI (CON = 31.5% ± 7.0% vs. RPC = 35.9% ± 6.6%; $P = 0.29$). Notably, more RPC-fed cows were pregnant by 150 DIM (CON = 56.8% ± 7.0% vs. RPC = 65.7% ± 6.8%; $P = 0.03$). For lactation up to 150 DIM, RPC supplementation improved fat-corrected milk yield (RPC = 46.3 ± 0.6 kg vs. CON = 45.2 ± 0.6 kg; $P = 0.02$), ECM yield (RPC = 45.4 ± 0.6 kg vs. CON = 44.3 ± 0.6 kg; $P = 0.02$), fat yield (RPC = 1.74 ± 0.2 kg vs. CON = 1.70 ± 0.2 kg; $P = 0.009$), and protein yield (RPC = 1.34 ± 0.2 kg vs. CON = 1.31 ± 0.2 kg; $P = 0.04$). These findings suggest that extended RPC supplementation benefits lactation, health outcomes, culling rates, and reproductive performance in dairy cows.

Key Words: fertility, estrus, survival

1547 Effect of a bovine appeasing substance (mBAS) on health of dairy cows. J. Rasia*, M. N. Marinho, A. S. Coelho, T. C. Santos, G. C. Evangelista, M. Perdomo, and J. E. P. Santos, *University of Florida, Gainesville, FL*.

The mBAS is a mixture of fatty acids resembling the composition of bovine pheromone that has been shown to attenuate responses to stress in cattle. Objectives were to evaluate the effects of a single application of a mBAS on pain and health in dairy cows. Cows ($n = 1,202$) were blocked by farm (1 to 3) and parity group (primiparous and multiparous) and assigned to a clustered randomized complete block design. Within farm, cohorts of primiparous and multiparous cows were assigned to receive 15 mL of either placebo (CON; 28 cohorts, 588 cows) or a mBAS (28 cohorts, 614 cows; FerAppease, FERA Diagnostics and Biologicals). Treatments were applied topically within 12 h of calving. Pain was assessed (attention, head, ear, and back positions, response to approach, and facial expression) at 4 and 14 DIM, and blood was analyzed for BHB concentrations at 7 DIM. Vaginal discharge was scored at 7 and 14 DIM. Diseases were diagnosed in the first 90 d postpartum. Continuous data were analyzed by linear mixed-effects models and categorical data by generalized linear mixed-effects models fitting a binary distribution and a logit link. Pain was analyzed by generalized linear mixed-effects models fitting a Poisson distribution and a log link. Cohort (1 to 56) was the experimental unit in all models. Models include the fixed effects of treatment (CON vs. mBAS), parity (primiparous vs. multiparous), farm (1 vs. 2 vs. 3), BCS at calving, calving problem (no vs. yes; dystocia,

twin, retained placenta, or stillbirth), and the random effect of cohort nested within treatment by farm and parity group. Treatment with mBAS reduced morbidity because of reduced risk of metritis in dairy cows.

Key Words: health, pain, pheromone

1548 Effects of peripartur supplementation of prototype postbiotics on intake, rumen fermentation, colostrum quality, and performance in transition dairy cows. S. Saikumar*, K. Arriola¹, A. A. Vargas¹, M. U. Siregar¹, N. Alfaro-Diaz¹, A. Barahona¹, S. Salvatierra¹, A. Cayetano¹, I. Yoon², and D. Vyasa¹, ¹University of Florida, Gainesville, FL, ²Cargill Animal Nutrition, Wayzata, MN.

This study aimed to evaluate the effects of prototype postbiotics on DMI, milk yield, milk components, BW, BCS, and rumen fermentation profile in transition cows from -35 d to 63 d postpartum. Sixty-two multiparous Holsteins, at 245 ± 3 d of pregnancy, were assigned to control (CON; $n = 31$) or postbiotics prototype (PB; $n = 31$) in a randomized block design. Diets were formulated based on CNCPS guidelines. Postbiotics prototype was top-dressed (25 g/d) on the TMR. For lactating cows, DMI, milk yield, and BW were recorded daily while milk samples were collected twice weekly for component analysis. Prepartum cows had BCS and BW measured weekly. Colostrum quality was measured using a Brix refractometer. Rumen fluid was collected on d -35, -14, and +14 for fermentation profile analysis. Data were analyzed using the GLIMMIX procedure in SAS using treatment, week, and their interactions as fixed factors, while cows nested in treatment was used as random factor. Significance was declared at $P \leq 0.05$. For prepartum cows, no effects of PB were observed on DMI ($P = 0.14$), BCS ($P = 0.84$), BW ($P = 0.59$), and total VFA ($P = 0.41$). However, postpartum DMI was greater (treatment × week; $P < 0.01$) during wk 7 and 8, and milk yield tended to be greater ($P = 0.10$) for cows fed PB. Energy-corrected milk yield was greater during wk 5, 7, and 8 (treatment × week; $P = 0.05$), while FCM tended to be greater (treatment × week; $P = 0.07$) for cows fed PB. No effects were observed on milk fat, milk protein, milk lactose, and MUN ($P > 0.05$). Fat concentration tended to be greater in colostrum for cows fed PB (+0.52% point; $P = 0.10$) but Brix score ($P = 0.49$) and colostrum protein ($P = 0.78$) were unaffected. In conclusion, PB prototype increased DMI, and milk yield after 6 wk, and increased ECM and FCM without affecting BW, BCS, or rumen fermentation profile. While PB supplementation tended to increase colostrum fat concentration, overall milk composition and prepartum measures were unaffected.

Key Words: postbiotics, transition dairy cows

Table 1 (Abstr. 1547).

Item (LSM ± SEM)	Treatment		AOR ¹ (95% CI)	P-value
	CON	mBAS		
Metritis, %	20.0 ± 2.2	9.2 ± 1.4	0.40 (0.27–0.60)	<0.01
Mastitis, %	4.2 ± 1.2	4.3 ± 1.2	1.02 (0.56–1.87)	0.95
Morbidity, %	25.5 ± 2.3	15.6 ± 1.8	0.54 (0.39–0.76)	<0.01
Left herd by 90 DIM, %	5.5 ± 1.4	4.1 ± 1.2	0.73 (0.32–1.65)	0.44
BHB, mM	1.05 ± 0.04	1.06 ± 0.04	—	0.86
BHB ≥ 1.2 mM, %	29.1 ± 4.8	28.3 ± 4.8	0.96 (0.51–1.80)	0.89
Pain score				
d 4	1.53 ± 0.12	1.32 ± 0.11	—	0.18
d 14	1.22 ± 0.10	1.32 ± 0.11	—	0.46

¹Adjusted odds ratio and confidence interval (CON as reference).

1549 Effects of lentinan supplementation on serum biochemistry, rumen microbiota, and rumen metabolites in transitional dairy cows. Y. H. Huang*, X. Li, S. J. Liu, and J. B. Cheng, *College of Animal Science and Technology, Anhui Agricultural University, Hefei, Anhui, China.*

The purpose of this experiment was to investigate the effects of lentinan (LNT) supplementation on oxidative stress, immunity, rumen microflora and rumen metabolites in dairy cows during the peripartum period. The study involved 20 transitional cows, which were divided into 2 groups: a control group (n = 10) and LNT group (n = 10, 40 g/d). The experiment began 21 d before the cows giving birth and continued for a duration of 42 d. Serum biochemical data were analyzed by *t*-test and interaction analysis, Welch analysis of rumen microbiota and metabolites and Spearman correlation. Compared with the CON group, the levels of serum antioxidant enzymes and immune globulin were significantly increased, and the levels of ROS, MDA and inflammatory factors were significantly decreased in the LNT group. At the rumen genus level, the addition of LNT significantly increased the abundance of *norank_f_Muribaculaceae*, *Shuttleworthia*, and *Ruminococcus*. Furthermore, the abundances of *Syntrophococcus*, *NK4A214_group* and *Candidatus Saccharimonas* were significantly reduced. Nontargeted metabolomics identified 276 upregulated metabolites and 246 downregulated metabolites. These metabolites were enriched in the leucine metabolic pathway. The results indicate that incorporating LNT into the diet can enhance the antioxidant capacity and immune response of dairy cows during the transition period while simultaneously reducing the inflammatory response. The underlying mechanism may be attributed to modifications in rumen microflora and alterations in rumen metabolites. These results may be achieved by upregulating *Shuttleworthia* and producing L-leucine and DL-phenylalanine.

Key Words: lentinan, rumen microorganisms, rumen metabolites

1550 Large-scale measured body weight changes effect on yield, health, reproduction, and feed efficiency in 3 different dairy cattle breeds. J. Lassen*^{1,2}, R. B. Stephansen², K. Kupisiewicz¹, and C.

Bengtson¹, ¹*Viking Genetics, Randers, Denmark*, ²*Center for Quantitative Genetics and Genomics, Aarhus University, Aarhus, Denmark.*

Daily BW predictions from a 3-dimensional camera system were recorded for Holstein, Jersey and Red Dairy Cattle after each milking. While individual BW measurements provide limited insights on their own, the continuous tracking of BW enables the assessment of BW changes during specific lactation periods, which are of critical importance. This study aimed to explore how BW changes in early and late lactation, the dry period, and days from calving until lowest weight after calving affected a cow's performance for various production traits (e.g., yield, reproduction, health, and feed efficiency). Data were available for a total of 9,142 cows in 19 herds: Jersey (2,513 cows), Red Dairy cattle (2,813 cows) and Holstein (3,816 cows). Mean BW was higher for Red Dairy Cattle (647.9 kg) and Holstein (683.8 kg) than Jersey (469.6 kg). Body weight changes were significantly related to various production traits. The analysis showed unfavorable effect of reduced BW in the dry period compared with lactation yield in the following lactation. Notably, cows that lost 25 kg during the dry period produced up to 8% less milk in the subsequent lactation compared with cows sustaining BW levels in the dry period ($P < 0.05$). Moreover, there is a positive effect on reproduction success as first inseminations of cows if performed in positive energy balance used 0.2 inseminations less per pregnancy compared with negative energy balance ($P < 0.05$). Body weight gain in late lactation was significantly related to BW loss in the dry period as well as to higher frequency of ketosis in the following lactation ($P < 0.05$). In general, the effects were similar across breeds both for the weight changes during lactation but also for the effects of weight changes on production traits. Weight loss in early lactation in percent of BW though was smaller for Red Dairy Cattle than for Holstein and Jersey. Also, the effect of weight change in late lactation affected feed intake in early lactation in the following lactation. For many traits there are substantial herd effects indicating both different management protocols but also different strategies and goals. This can and will lead to local truths, leading to improved management.

Key Words: weight changes, 3-dimensional images, production traits

Ruminant Nutrition 6: Calves and Heifers

1551 Does fiber make a healthy gut? The case of providing forage for calf diarrhea. G. D. Plaughner*, I. L. B. Fernandes, B. J. Bone, and M. C. Cantor, *The Pennsylvania State University, University Park, PA.*

In this randomized control trial, we evaluated the effects of feeding pelleted hay to pair-housed dairy calves on diarrhea characteristics (severity, duration, and cases). Holstein heifer calves ($n = 32$ pairs; 64 calves) were paired (5 ± 3 d of age) and randomly assigned in blocks of 4 to a control (CON; no hay) or treatment (HAY; pelleted timothy). All calves received 7.4 L/d of milk replacer (22% CP, 20% fat). Three trained observers with an interobserver agreement of $\kappa = 0.90$ recorded fecal score (0 = solid, 1 = loose, 2 = loose and sifts through bedding, 3 = watery) until 30 d of age to assess for diarrhea. Pairs with diarrhea had at least one calf with a fecal score = 2 for 2 consecutive days, or a score = 3 for 1 day. Severe diarrhea was a fecal consistency = 3 for at least 3 d in a pair. A case of diarrhea ended after resolution of diarrhea for 4 consecutive days. We used simple logistic regression models to assess the association of HAY with the odds of diarrhea severity, adjusting for pair average serum total protein (STP) and pair average birthweight (BWT), and season. We used multinomial logistic regression models to assess the association of HAY with the odds of diarrhea duration (0, ≤ 4 d, or ≥ 5 d) and number of diarrhea cases (0, 1, 2+), adjusting for STP, BWT, season, and antibiotic treatment for diarrhea, with block as a random effect. We had 27 pairs with diarrhea (14 = HAY, 6 = severe; 13 = CON, 10 = severe). Control calves were 5.05 times more likely to have a severe case of diarrhea than HAY calves (odds ratio [OR] 5.05; 95% CI 1.45–17.60; $P = 0.01$). There was no association of HAY with the odds of diarrhea duration (OR 0.33; 95% CI 0.09–1.20; $P = 0.09$) or multiple cases of diarrhea (OR 3.8; 95% CI 0.85–16.99; $P = 0.08$). We suggest that offering timothy hay pellets reduces the severity of diarrhea in pair-housed dairy calves.

Key Words: health, hay, social housing

1552 Evaluating the microbiome profile of neonatal calves supplemented with galacto-oligosaccharide using shotgun metagenomics. K. A. Ike*, D. P. Casper^{1,2}, U. Y. Anele¹, and M. F. Scott³, ¹North Carolina Agricultural and Technical State University, Greensboro, NC, ²Casper's Calf Ranch, Freeport, IL, ³Actus Nutrition, Eden Prairie, MN.

Galacto-oligosaccharide (GOS) is a prebiotic derived from whey. This study investigated the effect of GOS inclusion rates on gut microbiome of neonatal calves using shotgun metagenomics. Eighty-eight newborn Holstein bull calves, aged 2 to 5 d, were blocked based on their initial body weight and randomly assigned to 1 of 4 treatments using a randomized complete block design. The treatments consisted of a 22:20 (crude protein:fat) amino acid-balanced milk replacer (MR) supplemented with GOS at levels of 0 g/d (control), 2 g/d, 4 g/d, and 8 g/d. Calves were fed 0.283 kg of MR in 1.9 L MR twice daily for the first 14 d. From d 15 to d 35, the MR amount increased to 0.43 kg in 2.84 L per feeding, given twice daily. From d 36 to d 42, calves received 0.43 kg in 2.84 L once daily, followed by weaning. The GOS supplementation rate remained consistent despite the increase in milk volume. Fecal samples were taken rectally on d 40 before weaning and stored at -80°C until they were sent out for metagenome sequencing. Quality control of the reads was done using FastQC, and taxonomic classification was performed using Kraken2. Statistical analysis of the microbial abundance data

was done using EdgeR. *Bacillota* (firmicutes) phylum was dominant across all treatments. Calves receiving 4 g/d had the highest relative abundance for *Bacillota* (firmicutes) phylum and the lowest for *Bacteroidota* phylum. The relative abundance of *Lachnospiraceae* family was higher in calves fed 2, 4, or 8 g/d compared with 0 g/d. Calves fed 4 g/d had higher relative abundance for the *Atopobiaceae* family, whereas calves supplemented with 2 g/d had higher relative abundance of the *Coriobacteriaceae* family. Compared with 0 g/d, calves fed 2, 4, or 8 g/d were differentially lower (false discovery rate < 0.05) for *Shigella*, *Clostridium*, and *Campylobacter* genera and *Escherichia coli*, which are known to cause diarrhea in calves. *Salmonella* was also differentially lower for calves fed 4 g/d. Supplementation with GOS also differentially lowered several viruses, including *Tunavirus* strains (bacteriophages). Results from this study suggest that GOS helped to shape gut microbiome, reduce pathogenic burdens, and promote microbial homeostasis.

Key Words: calves, galacto-oligosaccharide, shotgun metagenomics

1553 Effect of calcified marine algae in calf starter, intake, and age on growth and rumen development in dairy calves. A. M. E. Hoving^{*1,2}, S. M. Kientz², S. G. Sayles², and A. H. Laarman², ¹Wageningen University & Research, Wageningen, Gekderland, the Netherlands, ²University of Alberta, Edmonton, AB, Canada.

Calcified marine algae (CMA) is used as a rumen buffer in cows, but effects on preruminant calves are unknown. The aim was to study effects of CMA in calf starter on growth and rumen development. Calves ($n = 24$) were individually housed, blocked by body weight, and randomly assigned in a 2×2 factorial arrangement of treatments. Starter was buffered with either 2.4% inorganic sources (limestone, bicarbonate, magnesium oxide; CT) or 1.25% CMA and 1.27% inorganic sources (limestone; CA). Daily, all calves were assessed for clinical health and received 6 L of milk replacer. Weaning transition occurred from d 49 to 63. Starter was fed ad libitum from d 15; intake was measured daily and body weight weekly. On d 51, rumen pH was measured continuously using an indwelling bolus. Harvest occurred either at 70 d of age (AGE) or whenever cumulative CS intake reached 50 kg (INT). At harvest, digesta and full and empty rumen and abomasum were weighed. Feces and rumen digesta were taken on d 42, 56, and 70 and at harvest. Data were analyzed in R using the LMER package and ANOVA test including diet and harvest point as fixed effect and arrival day as random effect. Before provision of calf starter at 15 d, CA calves had more sick days than CT ($P < 0.01$). After calf starter provision, there were no differences in sick days ($P = 0.10$). Rumen pH tended to be lower in CA groups ($P = 0.07$), but fecal pH was unaffected ($P = 0.29$). There were no differences among treatment groups of either total rumen or fecal VFA concentration ($P = 0.11$; $P = 0.11$). Empty rumen weight was higher for INT compared with AGE ($P < 0.01$), with no differences in digesta weight ($P = 0.84$). Full abomasum weight was higher for INT ($P = 0.02$), and CA-INT had higher abomasum weight than CA-AGE ($P < 0.01$). Rumen papillae length was shorter in CA calves ($P = 0.04$); papillae width was unaffected ($P = 0.91$). Despite more sick days before calf starter intake, CMA did not decrease calf starter intake ($P = 0.49$) or growth ($P = 0.12$). Further work is needed to evaluate how CMA may affect calf productivity depending on health status, but CMA may delay rumen development.

Key Words: rumen development, calcified marine algae, rumen

1554 Sialyllactose supplementation in milk replacer enhances growth performance via modulation of intestinal microecological development in preweaning dairy calves. W. Guo^{*1,2}, Y. Huang^{1,2}, Y. Cheng¹, S. Liu^{1,2}, S. Li^{1,3}, M. Ma², and Z. Cao^{1,2}, ¹State Key Laboratory of Animal Nutrition and Feeding, Beijing, China, ²International Calf and Heifer Organization, Beijing, China, ³College of Animal Science and Technology, China Agricultural University, Beijing, China.

This study aimed to evaluate the effects of sialyllactose supplementation in milk replacer on growth performance, diarrhea frequency, and intestinal microbiota composition in preweaning dairy calves. Forty-eight newborn Holstein dairy calves (44.09 ± 3.43 kg) were randomly allocated into 2 groups, receiving milk replacer with (MSL, $n = 24$) or without (CON, $n = 24$) sialyllactose supplementation from d 1 to d 14. The daily sialyllactose dosage was 11.12 g per calf. All calves were given commercial colostrum replacer within 1 h after birth, and then moved into individual hutches. Calves were fed 2.5 L (d 1 to d 7), 3 L (d 8 to d 14), or 3.5 L (d 15 to d 50) of milk replacer 3 times daily and weaned by d 56. The milk replacer powder was mixed with warm water ($\sim 42^\circ\text{C}$) at a ratio of 1:6.5 and provided via buckets. Body weight was measured at d 1, 14, 28, and 56, and feces were collected. Data on starter feed intake and fecal scores were recorded daily. Data were analyzed using PROC MIXED and GLIMMIX in SAS 9.4, with treatment, time, sex, and their interactions as fixed effects, and calf as a random effect. The results indicated that sialyllactose supplementation increased average daily gain from d 1 to d 56 ($P < 0.01$) and starter dry matter intake from d 3 to d 56 ($P < 0.01$) in calves. Diarrhea frequency in the MSL group tended to be lower than in the CON group from d 1 to d 56 ($P = 0.06$). Additionally, sialyllactose supplementation altered the fecal metabolic profile of calves at d 28 and d 56, with nucleotide metabolism and ABC transporter pathways significantly upregulated at d 28, and tryptophan metabolism and taste transduction pathways significantly upregulated at d 56. In fecal microbiota, the α diversity of the MSL group was higher than that of the CON group at d 14 ($P = 0.04$) and d 28 ($P < 0.01$). The β diversity differed between the groups at d 14 ($P = 0.07$), d 28 ($P = 0.06$), and d 56 ($P = 0.04$). Redundancy analysis showed that fecal microbiota was correlated with fecal metabolic profiles, with the potentially beneficial taxa *Bifidobacterium choerinum* enriched in the feces of the MSL group at d 28. In conclusion, early-life sialyllactose supplementation could serve as a nutritional strategy to enhance calf growth and health during the preweaning period.

Key Words: sialyllactose, milk replacer, intestinal development

1555 Impact of early-life milk secretory immunoglobulin A (SIgA) on ruminal microbial colonization in dairy calves. B. J. Schrag*, D. de Oliveira, M. Perdomo, Y. Zhai, K. C. Jeong, and F. Batistel, University of Florida, Gainesville, FL.

Rumen microbial composition and activity influences ruminant production efficiency. In nonruminants, milk secretory immunoglobulin A (SIgA) promotes commensal bacterial colonization and modulates gut microbiota, leading to long-term benefits. We hypothesized that early-life milk SIgA supplementation would enhance rumen microbial colonization. Forty-five female Holstein calves were blocked by birth date and randomly assigned to 1 of 3 treatments: (1) milk replacer (CON), (2) milk replacer with 7.2 mg of milk SIgA per day (SIgA), or (3) milk replacer with 7.2 mg of milk SIgA per day plus fresh rumen fluid from adult dairy cows (RSIgA). Treatments were administered from 2 to 28 d of age. Rumen samples were collected on d 4, 14, 28, 56, and 180. Data were analyzed using a mixed model, with treatment, time, and their interaction as fixed effects and block as a random effect. During the treatment period, there was a treatment $\times$ day effect on the Chao1 index (richness), with RSIgA and SIgA calves showing greater microbial richness at d 28 compared with CON ($P = 0.04$). Additionally, a treatment $\times$ time interaction was observed for the phyla *Bacteroidota* and *Verrucomicrobiota*. RSIgA reduced *Bacteroidota* relative abundance over time, whereas SIgA and CON increased it ($P = 0.03$). Meanwhile, *Verrucomicrobiota* relative abundance was higher at d 4 and 28 in RSIgA and SIgA calves compared with CON ($P = 0.01$). A treatment effect was also observed for the Shannon index (diversity), with RSIgA calves exhibiting greater diversity than SIgA and CON calves during the treatment period ($P < 0.01$). Moreover, *Fibrobacterota* relative abundance was lower in SIgA calves, followed by RSIgA and CON ($P = 0.05$), whereas *Proteobacteria* relative abundance was lower in RSIgA calves, followed by SIgA and CON ($P < 0.01$). During the carryover phase, the Shannon index was highest in RSIgA calves, followed by SIgA and CON calves ($P < 0.01$). Additionally, a treatment $\times$ time interaction was observed for *Euryarchaeota* and *Spirochaeta*, as their relative abundance decreased over time in CON calves but increased in RSIgA and SIgA calves ($P < 0.01$). Our preliminary results suggest that milk SIgA may modulate rumen microbial colonization.

Key Words: immune system, microbial community, rumen

ADSA Interdisciplinary Symposium: Design and Analysis of Experiments

1556 Survival bias in dairy farm data analysis. S. W. Eicker^{*1}, M. W. Overton², and D. V. Nydam³, ¹*Valley Ag Software, Tulare, CA*, ²*Zoetis, Parsippany, NJ*, ³*Cornell University, Ithaca, NY*.

In this era of massive computing power and “big data,” it is much easier to find connections or associations between various data variables. There has been a great deal of activity and many promises to revolutionize the management of dairy cows. Association often allows prediction of a future event. That is, when the first (explanatory) variable occurs, the second (outcome) variable appears. However seductive these might be, even from a biological perspective, it is crucial to remember that association is not causation. At least 2 issues impact this error. (1) There might be a third factor (often unknown or unobserved) that influences both variables. (2) Survival bias distorts the association between one factor and a second factor. Survival bias is a form of selection bias. Survivor bias occurs when researchers focus on individuals, groups, or cases that have passed some sort of selection process while ignoring those that did not, and can lead to incorrect conclusions due to studying only a subset of the original population. Unfortunately, this is very common in dairy data, since a percentage of cows are replaced every year, and the reasons for the replacement are often related to either the explanatory variable or the outcome variable. Survival bias has become so ingrained in our lives that we accept many of our associations as dogma. Two examples follow. (1) “Calf Pneumonia Has No Effect on Lactational Performance”: A multi-herd study was performed to examine the relationship between calf pneumonia and first-lactation milk yields. Stratification of milk weights by presence of calfhood pneumonia vs. healthy calves found no association. (2) “Older Cows Produce More Milk”: Cattle are replaced for many reasons, but low milk yield is an important factor. As a general rule, many low-producing cows do not get an opportunity to stay for another lactation. Cows in later lactations have been selected for more milk. If one creates a cohort of all the cows that first freshened in a given time frame, a different conclusion emerges. That cohort produces the greatest milk in the first lactation and less in every successive lactation.

Key Words: big data, survival bias

1557 Revisiting meta-analytic data analysis strategies for dairy science research. R. J. Tempelman*, *Michigan State University, East Lansing, MI*.

Meta-analysis studies continue to be published in the *Journal of Dairy Science* (JDS), with much of the corresponding analysis techniques developed by knowledgeable dairy scientists. These techniques have served the JDS research community well with respect to the determination of overall estimated effects or slopes and their standard errors. However, a recent JDS simulation study demonstrated these techniques to be suboptimal compared with more widely used likelihood-based techniques developed by statisticians. Most importantly, these methods can be used to provide prediction intervals (PI) on treatment effects. These PI are rarely reported in JDS but are of critical relevance to extension efforts, as they best reflect a plausible range of treatment effects within any one environment or future study. Although software such as the R package metafor is available for adapting these more optimal techniques, it is rather easy to modify existing mixed model software in R and in SAS to accomplish the same meta-analyses. I focus on adaptation of these techniques for Latin square designs, where it is important to extract standard errors of mean differences (SED) rather than the standard

errors of the means (SEM), even though SEM have been routinely used for meta-analyses by many dairy science researchers. Although SED are rarely reported in component Latin square studies, treatment mean separation information based on, for example, Tukey's test can be used estimate SED. Nevertheless, this strategy has limited effectiveness if only a few (i.e., 2–3) treatments are compared within component studies. Hence, reporting SED or estimated variance components should be routinely encouraged in future JDS papers to facilitate more accurate meta-estimates and PI in subsequent meta-analyses

Key Words: Latin square design, prediction interval, standard error

1558 Designing animal experiments with adequate statistical power using “pwr4exp”: An R package for robust research planning. K. Wang*, U. Arshad, and M. Niu, *Department of Environmental Systems Science, Institute of Agricultural Sciences, Animal Nutrition, ETH Zürich, Zürich, Switzerland*.

Effective experimental planning in animal research necessitates comprehensive power analysis to ensure both scientific reliability and adherence to ethical standards. Yet, an analysis of 4,376 nutrition studies published in the *Journal of Dairy Science* from 1995 to 2023 indicated a significant gap: only 4.73% explicitly mentioned conducting power analyses, and among these, 59% omitted essential methodological details. The majority of studies relied on proprietary software such as SAS. Although numerous open-source power analysis tools are currently accessible, their limited adoption indicates potential shortcomings in versatility and ease of use across diverse experimental frameworks. SAS methodologies (e.g., Stroup, 1999; 2002) provide flexibility but often lack automated approximation of degrees of freedom (df), complicating their application for complex models involving covariance adjustments. To bridge these methodological gaps, we developed pwr4exp, an open-source R package (available on CRAN: <https://cran.r-project.org/web/packages/pwr4exp/index.html>) specifically designed for model-driven power analysis. Leveraging linear mixed model frameworks, pwr4exp automates df approximation and accommodates complex study designs, including randomized block, split-plot, Latin square, and more flexible arrangements. It enables precise and streamlined calculations for main effects, interactions, and contrasts, enhancing decision-making on logistical constraints such as prioritizing key research questions in large-scale commercial herd trials or optimizing sampling protocols in smaller, intensive studies. Rigorous benchmarking against established SAS procedures confirms the accuracy and reliability of pwr4exp, particularly in resolving df approximation issues. In summary, pwr4exp significantly enhances the ease, transparency, and efficiency of experimental design planning in animal nutrition research. In our ADSA presentation, we will demonstrate pwr4exp through validated case studies and highlight its practical application as a critical component of experimental design in animal research.

Key Words: power analysis, experimental design, pwr4exp

1559 Associations between days open and dry period length vs. milk production, replacement, and fertility in the subsequent lactation in Holstein dairy cows with genomic test results. M. Overton^{*1} and S. Eicker², ¹*Zoetis, Blairsville, GA*, ²*VAS, King Ferry, NY*.

A retrospective, observational approach was used to investigate the association between genomic predictions, previous lactation days

open (PDOPN), and dry period length (PDDRY) with survivability, production, and reproductive performance in the subsequent lactation. Animal-level data ($n = 37,318$ cows) from 39 intensively managed, year-round calving US Holstein herds that used genomic testing were collected. Average PDOPN was 114 d (range 40–325) and PDDRY was 58 (range 20–120). Response outcomes had a common endpoint of 90 DIM in the subsequent lactation. Cumulative milk production (CUM90) was analyzed using a linear mixed model. Proportion replaced and proportion pregnant were analyzed using multivariable logistic regression. Explanatory variables included previous lactation 305-d mature equivalent milk (P305ME), PDOPN, PDDRY, previous lactation parity (PLCTGP 1, 2, or 3+), calf (female, male, or twins), number of clinical mastitis (NMAST) or clinical lameness (NLAME) events recorded in the previous lactation (0, 1, or 2+ cases), a genomic metric (Dairy Wellness Profit Index, DWP\$), and 2-way interactions. Interactions were retained with $P < 0.15$, and significance was declared with $P < 0.05$. Risks were calculated from the log-odds derived from the regression parameter estimates. Higher P305ME and DWP\$ were associated with lower replacement risk. Greater replacement risk was found with higher PLCTGP, more mastitis, and twins, and the effect of PDDRY was curvilinear, with shortest and longest PDDRY associated with highest replacement risk. Increasing DWP\$, P305ME, and PLCTGP were associated with greater CUM90. Higher PDOPN and twins were associated with lower CUM90. The PDDRY relationship was curvilinear, with shortest and longest PDDRY associated with reduced CUM90. Increases in DWP\$ were positive for pregnancy risk, but increasing PDOPN, PDDRY, P305ME, PLCTGP, disease, and twins were all associated with decreased pregnancy risk. Higher DWP\$, earlier conception, and a traditional dry period length were associated with higher milk production, improved reproduction, and reduced replacement risk.

Key Words: days open, dry period length, Dairy Wellness Profit Index (DWP\$)

1560 Associations between days open and dry period length vs. milk production, replacement, and fertility in the subsequent lactation in Holstein dairy cows. M. Overton^{*1} and S. Eicker², ¹*Zoetis, Blairsville, GA*, ²*VAS, King Ferry, NY*.

A retrospective, observational approach was used to investigate the association between previous lactation days open (PDOPN) and dry period length (PDDRY) with survivability, production, and reproductive performance in the subsequent lactation. Animal-level data ($n = 109,374$ cows) from 60 intensively managed, year-round-calving US Holstein herds were collected. Average PDOPN was 114 d (range of 40 to 325) and PDDRY was 58 (range of 20 to 120). Response outcomes had a common endpoint of 90 DIM in the subsequent lactation. Cumulative milk production (CUM90) was analyzed using a linear mixed model. Proportion replaced and proportion pregnant were analyzed using multivariable logistic regression. Explanatory variables included previous lactation 305-d mature equivalent milk (P305ME), week-36 milk (WM36), PDOPN, PDDRY, previous lactation parity (PLCTGP 1, 2, or 3+), calf (female, male, or twins), number of clinical mastitis (NMAST) or clinical lameness (NLAME) events recorded in the previous lactation (0, 1, or 2+ cases), and important 2-way interactions.

Interactions were retained with $P < 0.15$, and significance was declared with $P < 0.05$. Risks were calculated from the log-odds derived from the regression parameter estimates. Increases in PDOPN were associated with higher risk of replacement, and the effect of PDDRY was curvilinear, with shortest and longest PDDRY associated with highest risk of replacement. Replacement risk increased with increases in PLCTGP, more disease, and twins. A female calf was associated with lowest risk. Higher P305ME was associated with lower replacement risk and with greater CUM90. Both NMAST and twins were associated with reduced CUM90. The lowest PDOPN and PDDRY were associated with the highest pregnancy risk. Twins and disease had large negative impacts on pregnancy risk. Increasing P305ME had a small negative association with pregnancy risk that was biologically insignificant. In conclusion, cows should be managed to conceive earlier rather than later and experience a traditional dry period length to improve the subsequent lactation milk production and reproduction, and to reduce the risk of premature replacement.

Key Words: days open, dry period length, early lactation performance

1561 Sample size and content range for calibration transfer on milk fatty acid analysis using Fourier-transform mid-infrared spectroscopy. M. Matamura^{*}, E. Abe, T. Mishima, T. Shibata, and M. Kondo, *Mie University, Tsu City, Mie Prefecture, Japan*.

In the analysis of milk components using Fourier-transform mid-infrared spectroscopy (FT-MIR), calibration models developed by one primary instrument tend to be transferred to secondary instruments. Although milk components have been analyzed using FT-MIR in many countries, studies on calibration transfer remain scarce. Thus, this study aimed to use a numerical simulation to evaluate the effect of sample size and content range for calibration transfer on milk fatty acid (FA) analysis using FT-MIR spectrometry. A total of 143 milk samples (i.e., 51 and 92 bulk tank and individual cow samples, respectively) were used in this study. Preformed FA (≥ 18 C) in milk was determined using FT-MIR spectroscopy (CombiFoss 7 DC, Foss) and the Fatty Acid Origin Package. Preformed FA in all milk samples was also measured using gas chromatography as a reference value. A total of 100 milk samples were used to correct the transferred calibration model, whereas 43 samples were used to assess the applicability after correction. To evaluate the effect of the sample size and the content range, the sample size ($n = 2$ to 95) was randomly selected 30,000 times from the samples for correction, and the correction value was calculated by slope and bias correction at each time. As a result, 3 samples with a wide content range exceeding 1.0 g/100 g milk were used to greatly improve the accuracy of the FA values. Due to the narrow range of preformed FA content in bulk milk collected in this study, samples with a wider range of 1.0 g/100 g milk from bulk tank milk could not be obtained. Individual cow milk was necessary to obtain this wide range of preformed FA content values. Regarding the narrow range of the FA content, more than 10 samples were required for accurate correction. Overall, we found that the sample size and content range of milk to correct the transferred calibration model affected the accuracy of FA determination using FT-MIR spectroscopy.

Key Words: calibration transfer, Fourier-transform mid-infrared spectroscopy, slope and bias correction

Breeding and Genetics 4: Genetic Selection for Mitigation and Adaptation Traits (Part 2)

1562 Predicting methane production in dairy cows using milk spectra and genomic data. G. Martinez-Boggio^{*1}, K. L. Parker Gaddis², L. M. James³, R. Appuhamy³, J. E. Koltes³, E. Sarmikaso-glou⁴, R. J. Tempelman⁴, M. J. VandeHaar⁴, R. L. Baldwin⁵, K. F. Kalscheur⁶, E. A. French⁶, H. C. Mantovani¹, H. M. White¹, K. A. Weigel¹, and F. Peñaaricano¹, ¹*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI*, ²*Council on Dairy Cattle Breeding, Bowie, MD*, ³*Department of Animal Science, Iowa State University, Ames, IA*, ⁴*Department of Animal Science, Michigan State University, East Lansing, MI*, ⁵*Animal Genomics and Improvement Laboratory, ARS USDA, Beltsville, MD*, ⁶*Dairy Forage Research Center, ARS USDA, Madison, WI*.

Enteric methane (CH₄) is a major source of greenhouse gas emissions from dairy farming. Routine recording of CH₄ emissions at commercial farms can be challenging and costly. Fourier-transform mid-infrared (FT-MIR) spectroscopy is a powerful phenotyping tool that offers high-throughput, low-cost, non-invasive, and real-time predictions. We aimed to evaluate the feasibility of predicting CH₄ production using different sources of data, including productive traits, FT-MIR, and genomic data. We used data from 826 mid-lactation Holstein cows enrolled in 23 trials (7 to 10 wk) at 4 research farms. Data consisted of daily CH₄ production, daily milk yield (MY), milk composition and milk spectra data from morning and afternoon milkings (2 to 4 times/wk), and body weight records (every 2 to 3 wk). All cows were genotyped with 68,472 SNP. Milk spectra data consisted of FT-MIR absorbances at 1,060 data points in the infrared wavenumber range from 5,008 to 925 cm⁻¹. Methane production was measured using GreenFeed. We evaluated 3 alternative models to predict CH₄ production using a Bayesian approach: model XB with cohort (36 levels combining trial and treatment), days in milk (50–200), MY, and BW^{0.75} as predictors; model S as model XB plus FT-MIR data; and model GS as model S plus genomic data. Model predictive ability was evaluated in cross-validation using 10-fold cross-validation (cv105) and leave-one-trial-out cross-validation (loto). The cohort was removed from all models for the loto approach. Model GS achieved the highest correlations between the predicted and observed CH₄ production (cv105: 0.86 and loto: 0.77), followed by model S (cv105: 0.72 and loto: 0.54) and model XB (cv105: 0.61 and loto: 0.33). Overall, the inclusion of FT-MIR and genomic data improved CH₄ predictions. Our findings support previous evidence that FT-MIR can be used to predict methane emissions in dairy cows, and this information can be used to optimize herd management.

Key Words: methane emission, Fourier-transform mid-infrared spectroscopy (FT-MIR), prediction

1563 Global Methane Genetics Initiative: Acceleration of genetic progress for reduced methane emission in ruminants. Y. de Haas^{*1}, R. B. Banks², H. Montgomery², A. Jarvis³, R. F. Veerkamp¹, and B. Gredler-Grandl¹, ¹*Wageningen University & Research, Animal Breeding and Genomics, Wageningen, the Netherlands*, ²*Global Methane Hub, Santiago, Chile*, ³*Bezos Earth Fund, Cali, Columbia*.

The Global Methane Genetics (GMG) initiative aims to reduce methane (CH₄) emissions from the single largest emitter in the agricultural sector: ruminant livestock. Genetic differences in CH₄ emission between animals can be identified and included in selection decisions to breed animals that emit less CH₄, while staying healthy and producing nutrient-

dense food. Several countries have started recording individual CH₄ emission in cattle and small ruminants on commercial farms, allowing for estimation of breeding values for lower CH₄ emissions. However, for routine implementation globally, the number of phenotypes is still too small and is limited to only a few populations around the world. The GMG initiative is a multi-million-dollar investment by the Global Methane Hub's Enteric Fermentation R&D Accelerator and the Bezos Earth Fund, coordinated by Wageningen University & Research. The overarching aim is to accelerate genetic progress toward low-CH₄-emitting ruminants. The primary goals are (1) to significantly expand recording of CH₄ emissions in cattle and small ruminants, together with genotypes, pedigree, and rumen microbiome; (2) to share and disseminate knowledge via global working groups; and (3) to develop standard operating procedures in partnership with the ICAR Feed and Gas Working Group. There is a portfolio of programs starting early 2025 and involving around 50 partners from academia, research organizations, cattle and small ruminant breeding organizations, and other related stakeholders. This large-scale phenotyping and genotyping effort aims to result in more than 100,000 animals phenotyped and genotyped for CH₄ emissions with sniffers, GreenFeed, portable accumulation chambers, and laser methane detectors, and around 20,000 animals with rumen microbiome information in more than 25 countries in the Global North and South. A global database hosting phenotype, genotype, pedigree, and microbiome information is currently being developed within GMG, enhancing data sharing across the world. In this presentation we will show the potential of genetic selection as a CH₄ mitigation tool, as will be implemented in the GMG-program.

Key Words: methane emission, genetic selection, global impact

1564 Genetic parameters for enteric methane traits and their relationship with milk production in Danish Holstein and Danish Jersey Cattle. H. Schneider^{*1}, R. K. Lajevardi¹, M. Bjerring², P. Løvendahl¹, M. S. Lund¹, T. M. Villumsen¹, and R. B. Stephansen¹, ¹*Center for Quantitative Genetics and Genomics, Aarhus, Denmark*, ²*Department of Animal and Veterinary Sciences, Tjele, Denmark*.

Introducing a selection index for enteric methane (CH₄) emissions in dairy cattle is a key focus in animal breeding. A sustainable design of breeding schemes implies a comprehensive understanding of the genetic architecture of new traits and their relationship with existing breeding goal traits. However, most research has thus far focused on Holstein (HOL) cattle, with limited insights into other breeds. Thus, this study aimed to estimate genetic parameters for enteric CH₄ traits, obtained from sniffers installed in 17 commercial Danish farms, and their genetic correlation with milk production traits. A set of 4,727 HOL and 1,900 Jersey (JER) cows with 652,183 (HOL) and 231,377 (JER) daily records of milk, fat, and protein yields, as well as CH₄ and CO₂ concentrations, and the CH₄:CO₂ ratio, was used. In general, heritabilities for CH₄ traits were higher for JER than for HOL: for example, h² = 0.27 (SE = 0.06, JER) vs. h² = 0.14 (SE = 0.03, HOL) for the CH₄ concentrations. For JER, genetic correlations were mostly not significant and in a low to moderate favorable direction, indicating lower emissions with higher milk production. In contrast, they were moderate to high and unfavorable for HOL. We speculate that this breed difference may be mediated by the level of feed intake. Moreover, a strong negative genetic correlation between the CH₄:CO₂ ratio and fat yield was found for JER (r_g = -0.62,

SE = 0.17), possibly indicating a relationship between these traits via the negative energy balance, as the characteristic mobilization of body fat reserves results in an increased fat yield but also higher CO₂ concentration in the breath. Overall, the results of this study reveal heritable CH₄ emissions, whose genetic correlation with milk production traits differs between breeds. This might be caused by metabolic differences (e.g., related to the energy balance and feed intake) but requires further clarification based on larger data sets.

Key Words: dairy cattle, methane emission, milk production

1566 Investigating mRNA isoform expression patterns associated with feed efficiency in Holstein cattle. F. Muhammad^{*1}, V. Asselstine¹, C. F. Baes¹, F. Miglior^{1,2}, F. S. Schenkel¹, and Á. Cánovas¹, ¹Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, ²Lactanet Canada, Guelph, ON, Canada.

Feed represents a major variable cost in dairy production, making feed efficiency (FE) a key breeding objective for maximizing profitability. Among FE metrics, residual feed intake (RFI; kg DMI/d) is a common parameter representing the difference between expected and observed DMI, accounting for variations in maintenance and production needs. Residual feed intake is influenced by genetic and environmental factors, with its moderate heritability underscoring the influence of diet and management. Advancements in genomic technologies, including RNA-sequencing (RNA-Seq), have improved the ability to identify the most feed-efficient (low-RFI) animals for selection programs. This study aimed to identify differentially expressed (DE) mRNA isoforms between low- and high-RFI Holstein cows fed both control (C) and high-concentrate (HC) diets. Publicly available RNA-Seq data (GSE92398) from the NCBI Gene Expression database were analyzed, comprising liver biopsy samples (100 paired-end bp) from 9 cows (n = 4 high-RFI, n = 5 low-RFI). Quality control and read alignment were performed using the CLC Genomics Workbench (v.23.0.4) mapped against the ARS-UCD 1.2.11 bovine reference genome (v.110). Differential expression analysis (false discovery rate [FDR] <0.05; |fold change| >2) identified 121 mRNA isoforms under the C diet and 279 under the HC diet, irrespective of RFI status. Among these, 54 mRNA isoforms were upregulated in high-RFI animals, whereas 173 were upregulated in low-RFI animals, highlighting key regulatory influences on FE. Functional analysis revealed that DE mRNA isoforms upregulated in low-RFI animals were primarily associated with RNA stabilization and the regulation of mRNA metabolism, whereas those in high-RFI animals were linked to cell cycle processes and nuclear division. Both groups showed enrichment in pathways related to organelle division and cellular replication (FDR < 0.05). Further investigations will characterize both novel and annotated mRNA isoforms and their associated pathways. These insights are expected to enhance breeding programs, reduce the industry's environmental footprint, and boost economic outcomes.

Key Words: functional genomics, RNA sequencing (RNA-Seq), mRNA isoforms, residual feed intake

1567 Genotype by diet interaction for residual feed intake in Holstein cows using random regression models. V. Ambike^{*1}, M. J. VandeHaar¹, K. L. Parker Gaddis², J. Burchard², F. Peñagaricano³, H. M. White³, K. A. Weigel³, R. Baldwin⁴, J. E. P. Santos⁵, J. E. Koltes⁶, and R. J. Tempelman¹, ¹Department of Animal Science, Michigan State University, East Lansing, MI, ²Council on Dairy Cattle Breeding, Bowie, MD, ³Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI, ⁴Animal Genomics and

Improvement Laboratory, ARS USDA, Beltsville, MD, ⁵Department of Animal Sciences, University of Florida, Gainesville, FL, ⁶Department of Animal Science, Iowa State University, Ames, IA.

Dry matter intake (DMI) and residual feed intake (RFI) are important traits with major implications for dairy cattle profitability and sustainability. Understanding whether different dietary compositions are responsible for any genotype by environment (G×E) interaction for these traits might be important for optimizing genetic selection. This study aimed to determine whether genetic parameters for DMI and RFI varied across a continuum of dietary components. We analyzed 28-d feed intake records on 1,727 Holstein cows from 5 US research stations. All ranges provided are expressed as 10th to 90th percentiles. Dry matter content ranged from 48.3% to 53.9%. Estimated heritabilities from an animal mixed model for DMI and RFI were 0.23 and 0.14, respectively. Random regression models were used to model genetic effects as linear functions of dietary fat (3.1% to 5.5% DM), crude protein (CP; 15% to 18.4% DM), starch (21.4% to 29% DM), and neutral detergent fiber (NDF; 26.6% to 32% DM). Dietary fat content was identified as a significant ($P = 0.012$) source of G×E for DMI. There was no evidence of a significant ($P > 0.05$) G×E for DMI involving other diet components or for RFI. The estimated heritability of DMI ranged from 0.37 at 3.1% fat to 0.10 at 5.5% fat. Although these findings are preliminary, they underscore the potential importance of precision feeding strategies in dairy cattle genetic evaluations.

Key Words: feed efficiency, genotype by environment, heritability

1568 Genetic analysis of feed efficiency in Holsteins: From calves to lactating cows. I. C. Hermisdorff^{*1}, B. O. Mankanjuola¹, A. G. R. Phillips¹, R. E. Jahnel¹, F. Miglior^{1,2}, C. F. Baes¹, and F. S. Schenkel¹, ¹Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, ²Lactanet, Guelph, ON, Canada.

The objective of this study was to understand the regulation of feed efficiency across different life stages in Holstein cattle. Genetic correlations between feed intake and feed efficiency in calves and cows were estimated using data from 792 calves and 708 first-lactation cows. Intake was measured as metabolized energy intake (MEI) in calves and dry matter intake (DMI) in cows. The MEI was recorded as weekly averages: wk 1–3 (MEI1), when calves received ad libitum milk replacer, and wk 7–9 (MEI2), when they accessed a concentrate feeder. The DMI of cows was recorded weekly from lactation wk 9 to 44. Feed efficiency was defined as residual intake—that is, difference between observed and predicted intake based on metabolic body weight and energy-corrected milk in cows (RFI), and metabolic body weight and average daily gain in calves (RMEI). The model also included birth year, season for both calves and cows, and trial participation for calves. The data set comprised 12,246 DMI/RFI and 2,424 MEI/RMEI records per period. A total of 443 animals had records in both life stages, 349 as calves only, and 265 as cows only. A multi-trait repeatability model was fit to the data using ASReml software to estimate the genetic correlations among the 6 traits (MEI1, MEI2, RMEI1, RMEI2, DMI, and RFI). The genetic correlations were moderate between MEI2 and both DMI (0.54 ± 0.15) and RFI (0.48 ± 0.21), but low and nonsignificant between MEI1 and both DMI (0.12 ± 0.15) and RFI (0.08 ± 0.20). The genetic correlation between RFI and RMEI1 was low and negative at -0.37 ± 0.27 , but not significant, whereas its correlation with RMEI2 was positive and moderate at 0.64 ± 0.34 . Weak and negative genetic correlations between calves' feed efficiency in their first month of life and cows' feed efficiency may reflect incomplete rumen development, which may explain the stronger positive associations estimated between

their feed efficiency in the second month of life and the feed efficiency as cows. However, further analysis with a larger data set is needed to confirm these results and fully understand the genetic mechanism controlling the feed efficiency traits in calves and cows.

Key Words: calf, cow, feed efficiency

1569 Genomic selection and key genetic markers for heat tolerance in tropical Holstein dairy cattle. P. Sumreddee^{*1}, J. Pongpeng¹, S. Buaban¹, N. Siriratanatanyakul¹, K. Lengnudum¹, P. Sungkhaprecha², Y. Masuda³, and P. Phakdeedindan⁴, ¹*Bureau of Biotechnology in Livestock Production, Pathum Thani, Thailand*, ²*Rajamangala University of Technology Thanyaburi, Rangsit Center, Pathum Thani, Thailand*, ³*Rakuno Gakuen University, Hokkaido, Japan*, ⁴*Chulalongkorn University, Bangkok, Thailand*.

Dairy farming in tropical regions, such as Thailand, faces significant challenges due to heat stress, which negatively affects milk production and reproductive performance. Genetic improvement for heat tolerance (HT) offers a sustainable solution to mitigate these effects. This study aimed to estimate genomic breeding values (GEBVs) for HT and identify key genetic markers associated with heat resilience in tropical Holstein (TH) dairy cattle. The data set included 382,531 first-lactation test-day milk yield records from 44,236 cows calving between the years 1993 and 2021. Pedigree data consisted of 87,021 animals, with 3,397 animals genotyped for 42,119 SNPs. Heat stress was quantified using the temperature-humidity index (THI), with the average THI over the 7 d before and on test day (THI_{D7}) as the environmental stress indicator. Genomic estimated breeding values (GEBVs) for HT were derived using single-step GBLUP with a reaction norm model, where the rate of milk yield decline under heat stress served as a proxy for heat tolerance. Weighted single-step GWAS was performed to identify genomic regions associated with HT, and the results were further integrated with whole-genome sequence (WGS) data from tropical Holstein cattle to pinpoint potential causal variants. Results showed that milk yield declined when THI exceeded 77, with an average reduction of 0.15 kg/d per unit increase in THI (95% CI: -0.18 to -0.12 kg). The accuracy of GEBVs for HT was 31%–37% higher than traditional genetic evaluations. Key genomic regions associated with HT included *TAT* (tyrosine aminotransferase), *ITGA5* (integrin subunit α 5), *NCK-APIL* (NCK associated protein 1 like), *PTK2* (protein tyrosine kinase 2), and *HPD* (4-hydroxyphenylpyruvate dioxygenase). Integration of WGS data with GWAS results facilitated the identification of putative functional variants, including missense mutations, that could enhance selection accuracy. The identified genetic markers provide a foundation for developing DNA-based selection tools, such as SNP panels or marker-assisted selection kits, offering practical alternatives to GEBVs for farmers aiming to enhance heat tolerance in their herds. However, additional field validation is necessary to confirm their accuracy and effectiveness in on-farm selection programs.

Key Words: genomic selection, heat tolerance, weighted single-step GWAS (wssGWAS)

1570 Problems and opportunities for implementing a genotype by environment genomic selection evaluation for US Holstein cattle with a focus on heat tolerance. B. Fragomeni^{*1}, G. Dutta¹, H. Schober¹, T. McWhorter², S. Biffani³, F. Tiezzi⁴, and A. M. Miles⁵, ¹*University of Connecticut, Storrs, CT*, ²*Council on Dairy Cattle Breeding, Bowie, MD*, ³*Animal Breeding, Bioinformatics and Biostatistics CNR-IBBA, Milan, Italy*, ⁴*University of Florence, Florence, Italy*, ⁵*USDA-AGIL, Beltsville, MD*.

Genotype-by-environment interactions (G×E) represent a major challenge in genetics evaluations due to reranking of animals across environments. The US national evaluation includes many geographical regions that represent different climates and management practices that could cause G×E interaction and result in a reranking of animals. Additionally, heat stress poses a major concern for the US dairy industry and contributes to significant economic losses every year. Many studies have demonstrated genetic variation for heat stress tolerance in the US Holstein population. However, heat stress and G×E are still not implemented in the US national genomic evaluation due to the complexity of models or unknown effects of the inclusion of those new traits. The goal of this study was to evaluate the impact of G×E in US Holstein on a national level, with a focus on heat stress. Data were provided by the National Cooperators Database managed by the Council on Dairy Cattle Breeding (CDCB) and by the Cooperative Dairy DNA Repository (CDDR). Data included records of more than 10 million cows, their pedigree information going back at least 3 generations, and sire genotypes. Access to this data set was the result of a collective effort from University of Connecticut, the USDA Animal Genomics and Improvement Laboratory, CDCB, and CDDR. Genotype-by-environment interactions were evaluated using variance components estimated under different environments and by the changes in ranking of bulls with more than 100 daughters. Heritabilities for first-lactation milk yield varied from 0.16 to 0.25 across 4 states, and genetic correlations were as low as 0.8. Results for a heat stress evaluation demonstrated further sire reranking and will be presented. Many G×E analyses resulted in numerical problems and slow convergence due to model complexity and the large number of observations. Implementing a national evaluation that accounts for G×E and heat stress is possible; however, the effects of such evaluations must be inspected before recommendations are made to stakeholders.

Key Words: genetic evaluation, heat stress tolerance

1571 Computational deconvolution of bulk RNA-seq data reveals skeletal muscle myofiber composition differences in prepartum Holstein cows with varying muscle reserves. R. P. Moreira^{1,2}, M. Vicari³, H. Mulim^{*2}, T. M. Casey², J. P. Boerman², H. Rehman², and X. Fu⁴, ¹*Federal University of Parana, Curitiba, Parana, Brazil*, ²*Purdue University, West Lafayette, IN*, ³*State University of Ponta Grossa, Ponta Grossa, Parana, Brazil*, ⁴*Louisiana State University, Baton Rouge, LA*.

Skeletal muscle composition may affect metabolic adaptations during the transition period of dairy cows. However, the characterization of myofiber composition in prepartum Holstein cows remains largely unexplored. Understanding these variations can provide insights into metabolic efficiency and physiological adaptations of transition-period dairy cattle. In this study, we applied computational deconvolution to bulk RNA sequencing (RNA-seq) data of longissimus dorsi (LD) muscle biopsies taken at -21 d prepartum to estimate the proportions of type I, IIA, and IIX myofibers in Holstein cows. The cows were classified into low muscle (LM; n = 10) and high muscle (HM; n = 10) groups, according to LD depth at -21 d prepartum (averages of 4.22 ± 0.36 cm and 5.89 ± 0.34 cm, respectively). Previous studies found that HM cows produced larger calves and more milk in the early postpartum period. Single-cell RNA-seq data from LD muscle of Brahman cattle was used as a reference for the deconvolution analysis performed using CIBERSORTx software. The Shapiro-Wilk test confirmed non-normal distribution ($P < 0.05$), so the Mann-Whitney test was used to detect statistical differences among groups. The results showed that LM cows had a higher proportion ($P < 0.05$) of type I myofibers (21%) than HM cows (17%), suggesting greater oxidative, fatigue-resistant fiber pres-

ence. Conversely, HM cows had more type IIA fibers (44% vs. 40%; $P < 0.05$), indicating a shift toward oxidative-glycolytic metabolism. The oxidative-glycolytic nature of type IIA fibers may support increased glucose metabolism and energy turnover, potentially underlying larger calf size and greater milk synthesis evident in HM cattle. No significant difference was observed for type IIX fibers (39% in LM vs. 38% in HM; $P > 0.05$). These findings suggest differences in muscle fiber composition

between LM and HM cows that may affect metabolic efficiency in the peripartum period. Understanding these metabolic variations may help optimize dairy cow management during the transition period.

Key Words: RNA sequencing (RNA-seq), metabolic adaptation, muscle metabolism

Extension Education

1572 Assessing feeding management practices on Maryland dairy farms: Identifying needs and opportunities. N. Suresh^{*1}, I. Coelho², J. Semler³, T. Fernandes⁴, and F. F. Cardoso¹, ¹*Department of Animal and Avian Sciences, University of Maryland, College Park, MD*, ²*Social Data Science Center, University of Maryland, College Park, MD*, ³*University of Maryland Extension, Boonsboro, MD*, ⁴*School of Animal Sciences, Virginia Tech University, Blacksburg, VA*.

The Maryland dairy industry has been continuously declining in recent years, with the number of dairy cows decreasing from 54,000 in 2010 to 40,000 in 2023. To better understand the challenges farmers face, a comprehensive needs assessment survey was distributed to all 293 registered dairy farms in Maryland (IRB no. 2181157-1). Responses were received from 56 farms. Statistical analysis was performed using R version 4.3.3 (2024). Key variables analyzed include perspectives on corn silage quality, familiarity with mycotoxins and *Clostridium*, and forage management practices. Quantitative factors such as herd size, milk production, and testing frequency were considered alongside categorical factors such as silo type, inoculant use, spoilage history, and storage monitoring. Additional factors included farm location, breed composition, interest in education, accessibility needs, and openness to new technologies. Based on principal component analysis, farms were classified into 4 distinct clusters. Cluster 1 (15 farms) and cluster 2 (7 farms) were significantly associated with the Jersey breed ($P = 0.02$). Cluster 3 (13 farms) showed contrasting interest in educational resources ($P = 0.018$ for no interest; $P < 0.01$ for interest). Cluster 4 (8 farms) was similar to cluster 3 but with a reversed emphasis on education interest ($P = 0.01$ for interest; $P = 0.02$ for no interest). Only 3 farms had over 1,000 head of lactating cows; most were under 200. All farms that were not exclusively grass-fed used corn silage, showing it to be the most popular dairy feedstuff in Maryland. All farmers rated the quality of their corn silage as good, very good, or excellent, with very good being the most common at 46%. Two-thirds of farms used inoculants in their silage. Most farmers believed there should be more affordable testing options for mycotoxins and *Clostridium* and are open to adopting new technologies for detection. These data provide insights into the Maryland dairy industry, farmer challenges, and management strategies, supporting efforts to enhance sustainability and profitability through targeted education and outreach.

Key Words: extension, needs assessment

1573 Needs assessment of dairy producers in California. R. Branco-Lopes^{*1}, J. M. Heguy², B. M. Karle³, D. F. R. Bruno⁴, and R. A. Black⁵, ¹*University of California Agriculture and Natural Resources, Cooperative Extension Tulare County, Tulare, CA*, ²*University of California Agriculture and Natural Resources, Cooperative Extension Stanislaus County, Modesto, CA*, ³*University of California Agriculture and Natural Resources, Cooperative Extension Glenn County, Orland, CA*, ⁴*University of California Agriculture and Natural Resources, Cooperative Extension Fresno County, Fresno, CA*, ⁵*University of California Agriculture and Natural Resources, Cooperative Extension Sonoma County, Santa Rosa, CA*.

The objective of this study was to identify the concerns and research and educational priorities of dairy producers in California. In April 2024, a needs assessment questionnaire was mailed to Grade A dairy producers in California ($n = 1,068$). The questionnaire comprised the

following sections: general information, concerns and obstacles facing dairy producers (ranked as very concerned, somewhat concerned, or not concerned), cooperative extension (CE) research and educational priorities (ranked as high, medium, or low priority), target audiences for CE outreach, and preferred information delivery methods. The survey had an 8.1% response rate ($n = 87$), with farms distributed across Northern California (19.0%), the Northern San Joaquin Valley (36.0%), and Greater Southern California (45.0%). Responding farms had an average herd size of 1,663 cows (range: 50–7,000). Production systems were predominantly conventional (82.0%), and 16.0% were organic; the remaining responses did not report the production system (3.0%). The top 5 concerns and obstacles were (1) milk price, (2) feed costs or availability of feeds, (3) labor cost, (4) environmental concerns or regulations, and (5) water quality or availability. The 5 highest-priority research topics were (1) environmental regulation, (2) milk quality, (3) reproduction, (4) water use efficiency, and (5) herd health issues. The 5 highest-priority educational topics were (1) milk quality, (2) environmental regulation, (3) reproduction, (4) water use efficiency, and (5) herd health issues. Most respondents indicated that dairy owners should be the primary audience (85.0%) for CE programs, with half including managers. Fewer producers recommended targeting allied industry (21.2%) or dairy employees (17.5%). Preferred information delivery methods included newsletter or magazine articles (75.0%), on-farm training and meetings (41.2%), and half-day (or shorter) meetings (40.0%). Most respondents indicated that dairy employees should receive information primarily through newsletter or magazine articles (75.0%), on-farm training and meetings (63.8%), and half-day (or shorter) meetings (54.1%). These results will guide the development of future CE dairy programs in California.

Key Words: dairy farmer, survey

1574 Dairy farm employees' perception of *Mooving Cows* as an educational tool. M. Ruiz-Ramos^{*1}, J. Van Os¹, N. Cook¹, D. Ledesma¹, R. Craddock¹, O. Abraham², and M. Brauer¹, ¹*University of Wisconsin–Madison, Madison, WI*, ²*University of Kentucky, Lexington, KY*.

Handling dairy cows can affect their welfare and milk production positively or negatively. Proper cow handling requires training. We created an educational video game, *Mooving Cows*, as an interactive bilingual (Spanish and English) tool for participants to learn and reinforce appropriate cow handling practices. Our study objective was to characterize employees' perceptions of the game and its usefulness for continuing education. Spanish- or English-speaking employees ($n = 25$) with adult cow-handling roles from 3 Wisconsin dairy farms were recruited to evaluate the game. On mobile tablets, participants answered a pregame questionnaire, played the game, and then answered a postgame questionnaire. The pre- vs. postgame questionnaires asked about perceptions of real-life animal handling and of the game, respectively, using 5-point Likert scales. Here we present descriptive summary statistics. In the pregame responses, most participants indicated they enjoyed working with cows (87.5% score 4–5, fairly to very enjoyable), found it easy to move them (64.0% 4–5, fairly to very easy), were good at doing so (75.0% 4–5, fairly to very good), and felt safe when working with cows (83.3% 4–5, fairly to very safe). After playing the game, most participants found the in-game feedback clear (20.8% score 2–3,

slightly to moderately clear; 79.2% 4–5, very to extremely clear), thought the virtual cows behaved realistically (4.2% 1, not at all; 45.8% 2–3, slightly to moderately realistically; 50.0% 4–5, very to extremely realistically), enjoyed the game (20% 1–2, very to somewhat unenjoyable; 4% 3, neither; 76.0% 4–5, somewhat to very enjoyable), were willing to play the game again in the future (8.0% 1, never; 48.0% 2–3, rarely to occasionally; 44.0% 4–5, frequently to all the time), and thought it would be useful for both inexperienced (8.3% 1, not at all; 25.0% 2–3, slightly to moderately useful; 66.7% 4–5, very to extremely useful) and experienced cow handlers (8.7% 1; 34.8% 2–3; 56.5% 4–5; same scale as previous question). Overall, Wisconsin farm employees found *Mooving Cows* enjoyable and potentially useful for new and experienced staff, suggesting that this game would be a relevant continuing education tool for the dairy industry.

Key Words: animal handling training, bilingual tool

1575 DairyBot: A retrieval-augmented generation chatbot for domain-specific advice to dairy producers. R. Neupane^{*1}, B. Shrestha¹, A. A. Rajan², J. Wang², H. Shenoy², and S. Paudyal¹, ¹*Department of Animal Science, Texas A&M University, College Station, TX*, ²*Department of Computer Science and Engineering, Texas A&M University, College Station, TX*.

The objective was to develop and deploy DairyBot, an innovative open-source chatbot that provides personalized advice to dairy farmers by synthesizing domain-specific recommendations from authenticated user-provided documents and structured agricultural knowledge. Leveraging a modular architecture, DairyBot processes various document formats, including PDFs, spreadsheets, and textual reports, using optimized algorithms for semantic coherence and context preservation. These algorithms employ techniques such as recursive text chunking, sliding window analysis, and token overlap to mitigate context fragmentation and ensure that relevant information is accurately captured. The system integrates user-selectable open-source language models, achieving fast inference times of 1 to 3 min per response based on selected large language model, and facilitates multimodal interaction through a user-friendly interface developed using React. This interface allows farmers to ask questions, upload documents, and receive feedback, while also capturing user queries, document uploads, and feedback for longitudinal analysis of query patterns and model performance. PostgreSQL logs chat sessions with timestamped metadata, enabling the analysis of user behavior and system performance over time. Empirical validation was performed across 100 domain-specific questions, generated from provided PDFs, and demonstrated 73% accuracy in providing actionable recommendations, with errors often due to ambiguities in user-provided documents or temporal mismatches. By combining scientific knowledge with practical farming experience, DairyBot addresses a critical challenge in agricultural artificial intelligence (AI), ensuring that recommendations are grounded in both theory and real-world practice. The system's open-source architecture invites community-driven improvement, enabling seamless integration of emerging techniques such as HyDE (hypothetical document embeddings) for query expansion, and future work will focus on federated learning deployments to preserve data privacy across farms and multi-agent simulation for stress-testing sustainability scenarios under climate uncertainty. Furthermore, DairyBot's modular design allows for easy integration with existing farm management systems and deployment in farm personal computers, enabling farmers to leverage the power of AI-driven decision support while minimizing disruptions to their existing workflows. Overall, DairyBot represents an important step forward in the development of AI-powered decision support systems for agriculture, offering a scalable,

flexible, and user-friendly solution for dairy farmers seeking to optimize their operations and improve their bottom line.

Key Words: chat interface, dairy AI decision support, large language model

1576 How is media consensus on greenhouse gas emissions from animal agriculture evolving? R. Kohn^{*} and A. Tiwari, *University of Maryland, College Park, MD*.

Artificial intelligence has drastically changed distribution of information across all sectors of society. Information about sources and impacts of greenhouse gas (GHG) emissions may affect research priorities, regulatory policies, and consumer choices. The authors monitored the changes in Google and ChatGPT search responses to questions pertaining to GHG emissions from animal agriculture annually for the past 5 years. For example, authors have repeatedly asked the question, “How much would the average US citizen decrease their greenhouse gas emissions in percentage by stopping consumption of meat?” The authors estimate the correct answer to be 0% to 3% based on USDA and EPA published estimates. In 2020, Google provided a top link to a sponsored article in *The Economist* titled: “How much would giving up meat help the environment?” The summarized response was, “Decreasing consumption of animal-based foods by 50% from the baseline would reduce diet-related greenhouse gas emissions by 35%, to 3.3 kg CO₂ equivalents per person per day. In July 2024, the same question returned a less accurate response on Google and ChatGPT. For example, ChatGPT reported, “In summary, the estimated percentage reduction in total greenhouse gas emissions in the US due to a complete shift to a vegan diet varies, but it is generally projected to be significant, ranging from approximately 28% to 50%.” In January 2025, ChatGPT briefly provided a more accurate summary response to that question, citing a study from 2017, but provided grossly inaccurate summary responses to slightly different questions, and reverted to inaccurate responses thereafter. These examples show that media have converged to an increasingly narrow range of inaccurate estimates, often misquoting reputable sources, fudging simple mathematical calculations, and usually grossly overestimating greenhouse gas emissions from animal agriculture, especially in relation to plant-based agriculture. The hierarchy of sources used by search engines is variable. As search and language models are increasingly controlled by a few algorithms, researchers, educators, and consumers need to be aware of search and artificial intelligence bias. Strategies for disseminating accurate information are needed.

Key Words: greenhouse gas emission, artificial intelligence, media bias

1577 Stochastic simulation identifying key profitability factors of farmstead Cheddar cheese operations. C. Zaring Weir^{*}, A. Rihn, and E. Eckelkamp, *University of Tennessee, Knoxville, TN*.

Cheddar is a widely produced cheese due to its versatility and popularity. Farmstead Cheddar operations (FSC) add value by processing raw milk into Cheddar cheese. Limited resources exist for startup, equipment, and maintenance costs and profitability. A decision support tool (DST) using stochastic simulations in Microsoft Excel and @Risk identified how key farm metrics, inputs, and Cheddar sale price affected profitability metrics, including net present value (NPV), payback period (PP), and internal rate of return (IRR). Farm metrics included herd size (1,260 ± 971 cows), production per day (27 ± 6 kg), and percent of milk processed (54% ± 26%). Inputs included labor rate (\$25 ± \$4/h), number of employees (7 ± 3), facility cost (\$1,112 ± \$196/m²), equipment cost (\$897,433 ± \$169,609), processing capacity (68 ± 30 CWT/h),

utilities (\$7 ± \$4/CWT), other costs (\$13 ± \$4/CWT), and Cheddar sale price (\$23 ± \$4/kg). Inputs used means and standard deviations in Southeastern US DHI records (n = 351), Tennessee farmstead surveys (n = 8), and pricing from 13 specialty Cheddar cheeses from 6 brands. Latin hypercube sampling with a Mersenne twister was used for 10,000 iterations with a fixed seed. The mean investment cost was \$1,444,032 ± 306,800. Payback period (10 ± 9 yr), IRR (73% ± 71%), and NPV (\$10,384,621 ± \$23,641,665) varied. In all, 37% of iterations were not profitable within 20 yr, 41% were not profitable within 10 yr, and of those that were not profitable within 10 yr, only 8% would be profitable in 20 yr. The primary profitability drivers included Cheddar price, herd size, and percent processing, and were positively correlated with NPV and IRR, resulting in a decreased PP. The lowest profitability drivers were labor, equipment, and processing facility costs. Key factors affecting FSC profitability that may provide processors with generalized information regarding investment decisions were identified. Although designed for Southeastern US dairy farmers considering processing Cheddars, the DST can be customized to fit different cheeses with exact farm specifications, providing personalized investment insights.

Key Words: value-added, cheese processing, dairy processing economics

1578 Lessons learned through collaborative development of environmental sustainability resources for farm advisors. E. Wynands^{*1}, L. Zehr¹, B. Hilshey², L. McClintock², and S. Roche¹, ¹ACER Consulting, Guelph, ON, Canada, ²Dairy Management Inc., Rosemont, IL.

The US dairy industry is working collectively to progress toward its 2050 environmental sustainability goals. Farm advisors (e.g., nutritionists, veterinarians, agronomists) play pivotal roles in adopting best practices, disseminating information, and supporting environmental sustainability. The objective of this work was to develop science-based resources on key dairy sustainability topics for farm advisors through a collaborative approach. Additionally, we aimed to qualitatively assess the lessons learned, with the goal of informing the development of future science communication resources. The topics covered animal health, enteric methane, environmental modeling, reducing emissions from feed production and manure storage, and managing soil phosphorus. Slide decks, handouts, and video recordings were developed for 11 topics. All resources were posted to a central hub website in 2024, Dairy Conservation Navigator (www.dairyconservation.org/learning-hub), which contains a comprehensive resource library. Subject matter experts (n = 17) with backgrounds in research, extension, and on-farm advising on environmental topics were engaged to co-develop the content and deliver recorded presentations. Lessons learned from the process highlighted the critical role of experts in ensuring resource accuracy and alignment with current science. A flexible engagement strategy was essential to accommodate varying schedules and levels of involvement. The collaborative development process emphasized the need for consensus on objectives and a clear understanding of audience needs. Establishing key messages early helped maintain focus and purpose for each resource. Given the complexity of the topics, close collaboration between the project team and subject matter experts was vital to balancing breadth and depth while maintaining the appropriate level of detail. The resources were designed to be downloadable and customizable, allowing advisors to tailor them as needed. This co-development approach successfully produced 33 simplified sustainability resources for dairy industry advisors, incorporating the latest scientific insights.

Key Words: communication, conservation, advisor

1579 Evaluating Ontario dairy farmers' perception of barriers associated with the uptake of education programs: A focus group study. H. S. Carter^{*1}, C. Ritter², J. E. Brindle¹, J. P. Roy³, S. J. LeBlanc¹, and D. L. Renaud¹, ¹University of Guelph, Guelph, ON, Canada, ²University of Prince Edward Island, Charlottetown, PEI, Canada, ³Université de Montréal, Montreal, QC, Canada.

Education programs for dairy producers are updated and offered frequently, but effective strategies to optimize participation remain understudied. Therefore, the objective of this qualitative study was to explore the barriers to participation in education programs among Ontario dairy farmers and to examine the role of their veterinarian in supporting and conducting these initiatives. Five 60-min focus groups (3–5 Ontario producers per group) were conducted in person, with 20 participants representing 18 farms with a median herd size of 94 (range 50–600) cows. The median age of participants was 37.5 years (range 21–68 yr), and all identified as male (65%) or female (35%). Through applied thematic analysis using NVivo 14 software, a systematic approach to identifying and interpreting patterns in qualitative data, 5 themes emerged: (1) effective outreach, (2) perception of education, (3) motivators for participation, (4) considerations for participation, and (5) role of the veterinarian. Overall, producers valued education programs for peer interaction, gaining insights, and reinforcing knowledge. To maximize outreach, producers emphasized combining traditional (e.g., flyers, in-person invitations) and modern advertising (e.g., social media). In-person education programs were preferred due to the value of peer learning, but offering education through multiple formats may help overcome barriers. Barriers shared included resource constraints (time, location, and finances) and intrinsic factors (willingness to learn, interest, and perceived applicability of the subject). Participants highlighted that, as trusted advisors, veterinarians play a key role in education; however, their effectiveness varies, and participants expressed the importance of veterinarians continually seeking education to enhance their expertise. Frequent and valuable support is vital for strengthening relationships, as the strength of the relationship directly influences the veterinarian's impact on farm. These findings can inform the design, development, and delivery of future education programs to optimize producer adoption and engagement.

Key Words: dairy producer education, participation barriers, focus group

1580 Knowledge transfer efforts for Canada's Lifetime Performance Index (LPI). H. Sweett^{*}, B. J. Van Doormaal, C. Jaton, and A. Fleming, *Lactanet Canada, Guelph, ON, Canada.*

National selection indexes, including the Lifetime Performance Index (LPI) and Pro\$, are often used by Canadian dairy farmers, breeders, and artificial insemination (AI) companies to achieve breeding goals. Since its introduction in 1991, the LPI formula has evolved with national breeding objectives, and in 2024 a process to update the LPI was initiated. The objectives were (1) to create 6 subindexes published on their own and included in the LPI, (2) to remove the mathematical formula in communications and express subindexes on a standardized scale, and (3) to indicate relative emphasis on traits included but focus on the expected response for correlated traits. Lactanet consulted with 4 breed associations and the Genetic Evaluation Board (GEB), which includes 7 farmers, 5 industry members, and 6 technical experts, to identify the subindex traits and weightings to create the ideal LPI for each breed. Before the April 2025 launch, extension efforts explaining the subindexes, expression, and expected response targeted farmers, breeders, and AI companies through webinars, infographics, articles, presentations,

communication campaigns, and website resources. The LPI subindexes include Production (PI) to maximize fat and protein yields, Longevity and Type (LTI) to enhance longevity favoring functional conformation, Health and Welfare (HWI) to improve disease resistance and welfare, Reproduction (RI) to improve female fertility and calving performance, Milkability (MI) to optimize milking efficiency, and Environmental Impact (EI) to reduce the environmental footprint. For Holsteins, relative weights on the subindexes are 40% PI, 32% LTI, 8% HWI, 10% RI, 5% MI, and 5% EI, and were decided with the GEB by considering breed goals and correlations with the old LPI. Each subindex is expressed on a standardized scale, with an average of 500 and a standard deviation of 100, allowing users to compare animals. The expected genetic gain in a 5-year period is also provided, to show how traits included in the subindexes and correlated traits are affected. The new LPI and extension efforts have increased understanding while providing a balanced response for important traits.

Key Words: lifetime performance index, subindex, expected response

1581 *CalfWays: The Australian dairy industry sustainable calf management roadmap.* A. S. Hancock^{*1}, L. Sundermann¹, and A. M. Schuster², ¹*Dairy Australia, Melbourne, VIC, Australia*, ²*Schuster Consulting Group, Dubbo, NSW, Australia*.

With increasing consumer scrutiny on the fate of non-replacement dairy calves, their management is an important social license issue, and therefore a priority for the global dairy industry. This is evident in the emergence of industry initiatives aimed at reducing early-life slaughter worldwide. In Australia, based on a recent industry analysis of available

data, approximately 1 in 3 calves are slaughtered at an abattoir before 30 d of age, and approximately 1 in 10 are euthanized on-farm before 24 h of age. Dairy Australia is supporting the Australian dairy industry through the development and implementation of *CalfWays*, a roadmap designed to enable the industry to achieve the goals that (a) all calves enter a valued market chain; and (b) viable calves are not routinely euthanized on-farm. *CalfWays* offers a comprehensive framework, the intent of which is to help the dairy industry to continue to meet evolving consumer expectations without sacrificing on-farm profitability, thus creating a more sustainable and economically viable future for the dairy and beef sectors. The roadmap was developed using an iterative co-design process with extensive consultation, including face-to-face and online workshops with 150 stakeholders from the dairy and red meat sectors, overseen by a representative reference committee. Within the 4 core themes of Stakeholder Commitment, Capability Building, Market Connections, and Fostering Partnerships, the roadmap provides strategies and tactics for the creation of robust and reliable value chains for Australia's non-replacement dairy calves, primarily through the development of beef-from-dairy markets. Launched in early 2025, *CalfWays* is now in the implementation phase, focusing on proactive collaboration and partnerships across the supply chain. The Australian dairy and beef industries' research and development corporations (Dairy Australia and Meat and Livestock Australia) will co-fund a full-time position over a 3-year period to develop and oversee the work plan for implementation of the *CalfWays* initiative. They will also be responsible for the recruitment of other potential supply chain partners, and the development of monitoring, evaluation, reporting, and improvement metrics.

Key Words: surplus calf, beef-on-dairy, social license

Forages and Pastures 1

1582 Lactation performance and methane intensity of Holstein cows fed diets containing alfalfa grass silage inoculated with a multi-strain inoculant. G. O. Omoruyi^{*1,2}, C. S. Ballard¹, J. R. Green¹, H. M. Dann¹, A. Barkley³, R. A. Scuderi³, and S. Y. Morrison¹, ¹William H. Miner Agricultural Research Institute, Chazy, NY, ²University of Vermont, Burlington, VT, ³Lallemand Specialties Inc., Milwaukee, WI.

Holstein cows (n = 50; 111 ± 23 DIM; 2.9 ± 0.9 lactations) were enrolled in a randomized, complete block design study with 1-wk covariate and 6-wk treatment periods to evaluate the use of a multi-strain inoculant (*Pediococcus pentosaceus*, *Lactobacillus buchneri*, and *Lactobacillus hilgardii*; 4.7 × 10⁵ cfu/g) for alfalfa grass silage (AGS). Treatments were a diet with untreated AGS (CON) or inoculated AGS (INOC). Chopped alfalfa grass mixture was ensiled by inoculant treatment in individual bunkers (19.8 m × 6.1 m × 2.0 m) for ~124 d before use. Alfalfa grass silage was sampled at wk 0, 2, 4, and 6 during the treatment period for enumeration of lactic acid bacteria, yeasts, molds, and aerobic stability evaluation. Diets contained 62% DM forage (41% AGS, 21% corn silage) with 15.2% CP, 31.8% NDF, and 26.3% starch. The AGS had similar nutrient profiles (34.4 vs. 34.7% DM, 17.2 vs. 17.0% CP, 51.0 vs. 50.7% NDF, and 68.9 vs. 68.5 30-h NDF digestibility, % NDF for CON vs. INOC, respectively) and fermentation profiles consistent with inoculant use. Cows were group-housed, fed individually 1×/d, milked 3×/d, milk sampled 6×/wk, and had access to GreenFeed. Silage data were analyzed in RStudio (R v 4.4.2) using a Kruskal Wallis test by time. Cow data were summarized by week and analyzed by ANCOVA (MIXED procedure of SAS v 9.4). The model contained fixed effects (diet, week, diet × week, block). Yeast count at wk 6 was lower (*P* < 0.01) and aerobic stability at wk 2, 4, and 6 was improved (*P* ≤ 0.05) for INOC vs. CON AGS. The DMI was not affected by diet, but ECM, fat, and true protein yields were greater for cows fed the INOC diet (Table 1). Methane (CH₄) production and intensity were not different, and yield was not different within week. This study confirmed the importance of using an inoculant for AGS to reduce yeasts, improve aerobic stability, and improve animal performance.

Key Words: silage inoculant, cow, efficiency

1583 Enhanced aerobic stability in grass, alfalfa, and corn silages through microbial inoculation. R. C. Amaral^{*1}, K. L. Witt¹, I. Hindrichsen¹, J. N. Joergensen¹, K. A. Bryan², V. Vrotniakienė³, and J. Jatkauskas³, ¹Novonesis, Lyngby, Denmark, ²Novonesis, Milwaukee, WI, ³Lithuanian University of Health Sciences, Baisogala, Lithuania.

Table 1 (Abstr. 1582). Study results

Item	Diet			P-value	
	CON	INOC	SE	Diet	Diet × Wk
DMI, kg/d	27.6	27.5	0.2	0.63	0.91
Milk, kg/d	47.8	49.5	0.4	<0.01	0.85
ECM, kg/d	50.8	53.5	0.6	<0.01	0.96
Fat, kg/d	1.89	2.02	0.03	0.01	0.71
True protein, kg/d	1.39	1.46	0.02	0.01	0.90
ECM/DMI	1.83	1.85	0.02	0.45	<0.01
CH ₄ , g/d	473	476	6	0.66	0.44
CH ₄ yield, g/kg DMI	16.88	16.52	0.20	0.24	0.04
CH ₄ intensity, g/kg ECM	9.25	8.92	0.13	0.11	0.76

Silage inoculants support silage preservation by improving fermentation, enhancing aerobic stability to reduce spoilage, while maintaining nutrient quantity and quality. This study evaluated the effects of a combination of *Lactococcus lactis* and *Lentilactobacillus buchneri* on the aerobic stability of different silages. The study was conducted using grass, alfalfa, and corn harvested at recommended maturity. Grass was wilted to 29.4% DM and chopped to 2–3 cm, alfalfa to 29.7% DM and chopped to 2–3 cm, and corn to 34.0% DM and chopped to 10 mm. Each crop was ensiled in 3-L mini silos, with 5 replicates per treatment, and stored at room temperature. Treatments included (1) control (C) with no inoculant, and (2) inoculant (*L. lactis* [DSM11037] and *L. buchneri* [DSM22501]; SiloSolve FC, Novonesis, Lyngby, Denmark) applied at 150,000 cfu/g of fresh matter. Silages were stored for 90 d and thereafter opened, sampled, and analyzed for chemical composition. Aerobic stability was assessed by measuring pH, yeast and mold counts, maximum temperature (°C), and hours until temperature was more than 3°C above ambient temperature. Statistical analysis used the GLM procedure in SAS (version 9.4), with significance set at *P* ≤ 0.05. In grass silage, inoculated silage had a higher DM content at silo opening (*P* < 0.001) and significantly reduced pH after 10 d of aerobic exposure (*P* < 0.001) compared with the control silage. Yeast and mold counts decreased, maximum temperature dropped (*P* < 0.001), and aerobic stability was 4-fold higher compared with control (*P* < 0.001). Alfalfa showed similar patterns, although DM content was similar between treatments (*P* > 0.05). Spoilage microorganisms decreased (yeast *P* < 0.05, molds *P* < 0.001), and aerobic stability improved (*P* < 0.05). In corn silage, the DM content was higher in inoculated silage (*P* < 0.001); pH and maximum temperature did not show differences between treatments (*P* > 0.05); and aerobic stability time improved 2 times in inoculated silage (*P* < 0.05). In conclusion, in this study, inoculation with *L. lactis* and *L. buchneri* enhanced aerobic stability and reduced microbial spoilage across all silages.

Key Words: silage inoculant, aerobic stability, microbial population

1584 Effect of drought on microbiome community and metabolite profiles in kikuyu (*Pennisetum clandestinum*). V. T. L. Tan^{*1,2}, B. Brito-Rodriguez^{2,3}, P. T. W. Wong¹, A. Singh¹, R. Trethowan^{1,2}, and K. L. Plett^{2,3}, ¹The Plant Breeding Institute, University of Sydney, Cobbitty, NSW, Australia, ²Dairy UP Program, Camden, NSW, Australia, ³Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries and Regional Development, Menangle, NSW, Australia.

Kikuyu (*Pennisetum clandestinum*) is a perennial grass widely used in grazing systems for beef and dairy production worldwide due to its rapid growth and adaptation to harsh conditions. Unfortunately, kikuyu poisoning is an important factor hindering its expansion in grazing systems. Occasionally, the grass becomes toxic and causes poisoning in grazing livestock, with cattle (8.9%–32% mortality rate) being more susceptible than sheep and goats. Poisoning has been consistently observed when rapid grass growth is triggered by rainfall or irrigation following a prolonged period of summer drought. Additionally, previous studies have suggested that *Fusarium torulosum*, an endophyte, may be a causal agent of kikuyu poisoning. This study aimed to examine changes in the fungal community and metabolite profiles in 3 kikuyu cultivars: a drought-resistant cultivar, a drought-susceptible cultivar, and Whittet, under “toxic” conditions. Six replicate pots of each kikuyu cultivar were subjected to 6 weeks of “drought” treatment (30% field capacity)

followed by 1 week of re-watering to field capacity to stimulate rapid growth. Control pots were maintained at 80% field capacity throughout the 6-week treatment period, followed by 1 week of watering to field capacity. After 1 week of re-watering, leaf, stem, root, and soil samples were collected for ITS sequencing and non-targeted metabolomics. Interestingly, although *Fusarium* was among the 20% most abundant genera, other notable fungal genera identified included *Sarocladium*, *Cladosporium*, *Sporothrix*, *Poaceascoma*, *Myrmecridium*, *Phialophora*, and *Podospora*. Results from this experiment could assist researchers in gaining better understanding of the microbiome and metabolite profiles in kikuyu under favorable conditions, as well as the changes that occur under unfavorable conditions. This knowledge could aid in identifying potential causal factors of kikuyu poisoning.

Key Words: kikuyu poisoning, endophyte, drought

1585 Climate-smart ruminant production through dietary manipulations in a climate-resilient environment. T. A. Arilekolasi^{*1}, O. B. Omotoso^{2,3}, and J. A. Alokun², ¹*Federal University Oye-Ekiti, Oye-Ekiti, Ekiti State, Nigeria*, ²*Federal University of Technology, Akure, Ondo State, Nigeria*, ³*Net Zero and Resilient Farming Rothamsted, North Wyke, UK*.

Ruminants contribute significantly to global warming through the emission of enteric CH₄. A farmer-friendly approach to ensuring climate-smart production is through dietary manipulations. This study was laid out in a 3 × 3 factorial arrangement to examine the effect of 3 fertilizer types and 3 drying methods on the nutritive quality and CH₄ production of *Cajanus cajan* hay as feed for ruminants. *Cajanus cajan* pasture was artificially sown on nitrogen-depleted tropical soil. Poultry manure (PM) was applied 2 weeks before planting at 3 t/ha, and urea was applied to a portion 2 weeks after planting (WAP) at 30 kg/ha. Another portion was established without fertilizer application (control plot). Forages were harvested at 12 WAP and subjected to 3 drying methods (air drying, sun drying, and solar cabinet drying) for hay production. Chemical analyses were carried out using standard procedures. The feed value of hay was determined using the in vitro gas production technique (IVGPT). Data collected were subjected to statistical ANOVA using the GLM procedure of Minitab 18, and means were separated using Tukey's (HSD) test at a 5% probability level. There were significant differences in the values obtained for proximate components, fiber fractions, anti-nutritional factor (ANF) composition (alkaloids, tannins, phytates), in vitro dry matter degradability (IVDMD), gas volume, and CH₄ production of the produced hay. Interactions of different combinations showed that unfertilized, sun-dried hay had the highest dry matter (DM) content (91.62%), whereas solar cabinet-dried, urea-fertilized hay had the highest crude protein (CP) content. However, the lowest CP was recorded in urea-fertilized, air-dried hay. Poultry manure air-dried hay had the least crude fiber (CF; 22.68%), whereas PM air-dried hay had the highest energy content (15.34 kJ/100 g DM), which was similar to the energy content of unfertilized sun-dried and PM sun-dried hay. Additionally, PM solar-dried hay had the lowest CH₄ production (3.66 mL/200 mg) and the highest organic matter digestibility (OMD; 41.96%). Thus, PM was the most efficient fertilizer applied, and the solar drying method improved the nutrient and feeding values of the hay. This approach could be adopted for hay-making as part of climate-smart feed strategies.

Key Words: *Cajanus cajan*, poultry manure, methane production

1586 Palatability of inoculated and uninoculated corn silage at 0 and 24 h post-defacing when fed to pregnant Holstein heifers. C. S. Ballard¹, A. E. Pape¹, J. R. Green¹, A. Barkley², R. A. Scuderi^{*2},

and M. Castex³, ¹*William H. Miner Agricultural Research Institute, Chazy, NY*, ²*Lallemand Specialties Inc., Milwaukee, WI*, ³*Lallemand SAS, Blagnac, France*.

The study objective was to evaluate palatability of corn silage treated with a multi-strain inoculant immediately and 24 h after defacing. Conventional corn hybrid was inoculated with water (control, C) or treated (T) with a multi-strain inoculant containing 1 homofermentative strain (*Pediococcus pentosaceus*) and 2 heterofermentative strains (*Lentilactobacillus buchneri* and *Lentilactobacillus hilgardii*) at 2.5×10^5 cfu/g forage. After ensiling for 189 d, spoilage was removed daily, and silage was dispensed into forage carts and stored out of sunlight at ambient temperature. Silage was sampled relative to experimental feeding period on d 0, 6, and 12, evaluated for aerobic stability, and enumerated for lactic acid bacteria, yeasts, and molds. Sixteen pregnant Holstein heifers were introduced to 1 of 4 corn silage treatments for 30 min over 4 d: freshly defaced control (C0), control after 24-h aerobic exposure (C24), freshly defaced treatment (T0), and treatment after 24 h of aerobic exposure (T24). A TMR was fed ad libitum for the remainder of the day. Palatability of silage treatments (C0, C24, T0, T24) was evaluated by offering each heifer 2 bins of different silage treatments for 3 h over a 12-d treatment period and measuring dry matter intake (DMI) after 30 min and 3 h. Silage characteristics were analyzed in R (v. 4.4.2) using Kruskal-Wallis within sampling time. Effects of silage treatments on intake were analyzed using the MIXED procedure in SAS v. 9.4. Relative palatabilities of each feed type were estimated via a paired comparisons analysis that considered a range of thresholds for proportional intake required to designate a feed type as preferred. Combined with 2 durations of feed availability (30 min and 3 h), palatability was estimated separately for each of 8 distinct configurations. Inoculation reduced yeasts on d 0 and 12 and improved stability on d 0. Both DMI and relative palatability at 30 min and 3 h ($P < 0.05$) were greater for freshly defaced corn silage (1.18 vs. 0.91 kg/30 min and 2.79 vs. 2.32 kg/3 h, respectively). There were higher levels of intake for T corn silage compared with C after 30 min (1.14 vs. 0.94 kg; $P = 0.01$). Under some configurations, inoculant appeared to have a moderate preservative effect, attenuating the decrease in palatability due to aerobic exposure.

Key Words: silage inoculant, palatability

1587 Revealing the real nutritional value for maize silage. M. R. Islam^{1,2}, M. Correa-Luna^{1,2}, C. E. F. Clark³, J. I. Gargiulo^{2,4}, and S. C. Garcia^{*1,2}, ¹*University of Sydney, Camden, NSW, Australia*, ²*DairyUP Program, Camden, NSW, Australia*, ³*Charles Sturt University, Wagga Wagga, NSW, Australia*, ⁴*NSW Department of Primary Industries and Regional Development, Menangle, NSW, Australia*.

Whole-crop maize silage (WCMS), a common forage feed for ruminants, is typically considered as one unit, but it is composed of both stover (forage) and grain fractions. Stover supplies fiber, and grain supplies starch; both fractions contribute to the total energy content of the feed. Although the starch content is almost fully digestible in the whole tract of cows and provides almost full energy, WCMS with varying starch has been reported to contain similar total metabolizable energy (ME). We reviewed over 217 studies published from the year 2000 onward to explore the reasons behind the similar energy contents in WCMS containing contrasting starch amounts and to reveal the real nutritional value of WCMS. When WCMS contained >25% starch, fiber digestibility was reduced, likely due to a decrease in rumen pH and the intolerance of fibrolytic bacteria to acidity. Conversely, amylolytic bacteria, which degrade starch, are more tolerant to low rumen pH, and any undigested starch in the rumen is almost completely digested in the intestinal tract

(98%–100%). As a result, the overall digestibility of WCMS remained relatively constant across different starch levels. To overcome this problem, we propose to assess WCMS through a 2-compartment (i.e., stover + grain) digestibility model. We tested this approach experimentally with WCMS from a field study evaluating plant density and harvest time of maize. Stover ME was calculated from the sum of the contributions of ME from leaf, stem, and cob structure, using an in vitro digestibility technique. Grain ME content was calculated from the proportion of grain and literature-reported total-tract digestibility of 98%. Both methods showed similar ME content across treatments (average of 10.7 ± 0.21 and 11.6 ± 0.11 , respectively), but the ME estimated through the 2-compartment model was 15% greater, realistically reflecting the nutritive value of WCMS. The 2-compartment approach opens new possibilities in remote monitoring of stover and grain, as well as the integration into mechanistic models to assist producers in making informed decisions regarding diet formulation, yield optimization, variety selection, and harvest timing.

Key Words: whole-crop maize silage (WCMS), stover, grain

1588 Understanding and predicting changes in nutritional value of Kikuyu. J. Insua^{1,2}, M. Correa-Luna^{2,3}, R. Islam^{2,3}, F. M. Hasan^{2,3}, M. Nikolic^{2,3}, J. Gargiulo⁴, P. Beale^{2,5}, and S. C. Garcia^{2,3}, ¹National University of Mar del Plata–CONICET, Mar del Plata, Buenos Aires, Argentina, ²Dairy UP Program, Camden, NSW, Australia, ³Dairy Science Group, School of Life and Environmental Sciences, Faculty of Science, University of Sydney, Camden, NSW, Australia, ⁴NSW Department of Primary Industries and Regional Development, Australia, Camden, NSW, Australia, ⁵Local Land Services (Hunter), Camden, NSW, Australia.

Kikuyu (*Cenchrus clandestinum*) is a high-producing C4 grass used by dairy farms in temperate-warm dairy regions of Australia, but its nutritive value declines rapidly. This study aimed to understand and predict short-term (daily) changes in the nutritional value of kikuyu pasture regrowth by modeling the morphophysiological aspects of plant tissue using the Morphological and Digestibility Pasture (MDP) model. The MDP model, originally developed for a C3 grass, uses daily temperature to drive plant morphology traits (leaf number and length) and fiber digestibility. The model was parameterized for kikuyu and evaluated with detailed data from controlled, plot-scale ($\sim 10 \text{ m}^2$ each) experimental periods conducted during the austral summer months of 2022, 2023, and 2024. In replicated plots, a randomly selected 1-m^2 pasture area was harvested at consecutive leaf-appearance intervals to measure herbage mass, plant morphology, neutral detergent fiber (NDF), and 24-h in vitro digestibilities of NDF (NDFD) and dry matter (DMD) of leaf blades. In parallel, leaf morphogenetic traits (appearance, elongation, and lifespan) were measured on marked tillers every 3 d. The MDP model, parametrized with data from the first 2 experimental periods, accurately estimated the changes in NDFD observed in the third experimental period, used for independent model evaluation. Root mean square error was 1.9%, $R^2 = 0.94$, and bias correlation factor was 0.80. The primary factor driving the decline in the nutritive value of kikuyu was the consistent loss of NDFD with leaf tissue aging ($R^2 = 0.77$). This leaf-aging effect explained the steady decrease in pasture DMD across all sampling periods ($R^2 = 0.66$; from 65% to 40%) and the strong negative observed relationship between NDFD and herbage mass ($R^2 = 0.63$), with NDFD reducing by 13 to 16 percentage units per ton of DM per hectare. This study (a) highlights the relevance of monitoring the thermal time of kikuyu regrowth period to prevent milk production losses due to nutritive value decline and (b) provides sound

evidence to include real-time modeling of morphophysiological aspects to manage pasture quality.

Key Words: fiber digestibility, leaf aging, modeling

1589 Machine learning increases the accuracy of satellite-derived pasture cover estimation. B. N. Azubuike^{*1,2}, A. Chlingaryan^{2,3}, C. E. F. Clark^{2,4}, and S. C. Garcia^{1,2}, ¹Dairy Science Group, School of Life and Environmental Sciences, Faculty of Science, University of Sydney, Camden, NSW, Australia, ²Dairy UP Program, Camden, NSW, Australia, ³Livestock Production and Welfare Group, School of Life and Environmental Sciences, University of Sydney, Camden, NSW, Australia, ⁴Gulbali Institute, Charles Sturt University, Wagga Wagga, NSW, Australia.

Accurate estimation of pasture cover is critical for effective farm management and feed budgeting. Satellite-derived indices such as the Normalized Difference Vegetation Index (NDVI), though widely used, often correlate poorly with biomass due to saturation effects with canopy closure, highlighting the need for a more efficient approach. Here, we aim to improve pasture cover prediction using machine learning and optimization techniques to develop a unified framework that integrates raw satellite data with paddock-specific and weather information, while evaluating alternative data processing methods to reduce prediction errors. A series of experiments were conducted using both non-interpolated and interpolated data sets, with the data split by farm. Outlier removal and data interpolation were applied to minimize gaps and temporal mismatches between plate meter and sentinel data sets. Hyperparameter optimization was performed using grid search and cross-validations to determine the optimal model configuration. Models were compared under several scenarios: one where models were built with and without standard vegetation indices (NDVI, EVI, SAVI, NDRE) to assess their contributions; another where interpolation was used to effectively enlarge the data set; and a third where the training data were partitioned into 4 subsets based on week-of-month for progressive training. Additionally, a farm-specific approach was implemented by reserving data from some farms for training and using data from others for testing to capture regional variability. The integrated framework explained 62% to 77% of the variability in pasture cover. Interpolation reduced the MAE from 289 kg DM/ha to 224 kg DM/ha, whereas progressive training achieved $\sim 30\%$ reduction in error. This experiment demonstrates that robust remote cover forecasting is achievable using advanced data manipulation and optimization techniques without heavy reliance on vegetation indices. The framework provides valuable insights for optimal model configuration and improved pasture cover estimates. Overall, the techniques significantly reduced prediction errors and suggest further enhancement potential through expanded data integration, real-time optimization, and inclusion of additional environmental variables.

Key Words: biomass prediction, machine learning, optimization

1590 The effect of grazing perennial ryegrass or perennial ryegrass-white clover swards under varying nitrogen fertilizer rates on dairy cow production. M. H. Bock^{*1,2}, Z. C. McKay², L. Delaby³, D. Hennessy⁴, and B. McCarthy¹, ¹Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland, ²University College Dublin, Belfield, Dublin, Ireland, ³INRAE, L'Institut Agro, UMR Pegase, Saint-Gilles, France, ⁴School of Biological, Earth and Environmental Science, University College Cork, Cork, Ireland.

A 3-year (2022–2024) rotational grazing study was undertaken to investigate how dairy cow production could be maintained by grazing a perennial ryegrass sward including white clover under a decreased rate of nitrogen (N) fertilizer application compared with a perennial ryegrass-only sward under a higher N fertilizer application rate. The 4 treatments were a perennial ryegrass-only sward receiving 150 kg N/ha (GO-150) or 225 kg N/ha (GO-225) annually and a perennial ryegrass-white clover sward receiving 150 kg N/ha (GC-150) or 75 kg N/ha (GC-75) annually. Lactating dairy cows were randomized and blocked into treatments by breed, parity, and calving date ($n = 112$). The stocking rate in each treatment was 2.56 cows/ha, which rotationally grazed 20 paddocks each year. Pregrazing herbage mass and quality, and sward clover content, were quantified weekly. Individual cow milk yield was recorded daily, and milk composition was analyzed once a week. Sward measurements were taken before and after grazing. Statistical analysis was conducted using SAS on milk data using mixed models with fixed effects including treatment, breed, parity, year, and treatment, paddock, block, and month for sward measurements. The cows grazing the GC-150 sward had a greater total milk solids (fat + protein) yield ($P = 0.004$; + 22 kg/cow) compared with the GO-225 cows. The GC-75 treatment had no significant difference for total milk yield ($P > 0.1$) compared with the 2 GO treatments. The GC-150 sward had the highest crude protein content ($P < 0.05$) compared with the 2 GO treatments. The results show that with the inclusion of white clover, cows can maintain production despite reduced N fertilizer application.

Key Words: milk production, sward quality, nitrogen inputs

1591 Nutritional effects of organic acids and physical properties of short-stature corn. H. G. Burchard^{*1}, N. Suresh¹, J. Semler¹, V. Malacco², T. Fernandes³, and F. F. Cardoso¹, ¹*Department of Animal Sciences, University of Maryland, College Park, MD*, ²*Kemin Industries, Des Moines, IA*, ³*School of Animal Sciences, Virginia Tech University, Blacksburg, VA*.

Whole-plant corn silage (WPCS) is an indispensable forage in a dairy cow's diet due to high energy content. However, the energy availability to the cow depends on the WPCS quality. This study aimed to assess the physical properties at harvest of 2 corn varieties, short-stature corn (STC) and conventional corn (CC), and evaluate the effect of organic acids on nutritional quality. The addition of a commercial organic acid blend (Silage SAVOR Plus Liquid, Kemin Industries Inc., Des Moines, IA), was used as the treatment (ORG) versus control (CON: water; no organic acids). Experimental silos were used to analyze samples at specific ensiling days (d 0, 7, 15, 30, 60, and 120). Samples from d 7, 15, 30, and 60 were sent to a commercial laboratory (Dairy One, Ithaca, NY) for analysis. Data were analyzed using the MIXED procedure in SAS. At harvest, the average component weights for STC and CC, respectively, were as follows: whole plant (2.43 and 2.40 kg), cobs (1.92 and 1.73 kg), leaves (0.75 and 0.84 kg), tassel (0.01 and 0.03 kg), and stalk (0.59 and 0.32 kg). The particle size distributions were 14% and 15% on the upper sieves, 56% and 58% in the middle, 15% and 17% on the lower sieves, and 13% in the pan for STC and CC, respectively. The DM percentage was greater ($P = 0.03$) for STC (30.0) compared with CC (29.0) and significantly greater ($P < 0.01$) for ORG (30.0) compared with CON (29.0). Percentage of CP was greater ($P < 0.01$) for CC (10.4) compared with STC (9.7), and greater ($P < 0.01$) for ORG (10.2) than CON (9.9). Neutral detergent fiber was greater ($P < 0.01$) for CC (35.2) compared with STC (30.4) and greater ($P = 0.01$) for CON (34.0) than ORG (32.0). Acid detergent fiber was greater ($P < 0.01$) for CC (19.6) compared with STC (17.1) and greater ($P < 0.01$) for CON (19.2) than ORG (17.4). Neutral detergent fiber digestibility over a 30-h period for d 60 did not differ among treatments. Starch was greater ($P < 0.01$) for STC (38.3) compared with CC (30.4), and no difference ($P = 0.65$) was seen between ORG and CON. There was no significant difference in pH levels ($P = 0.09$) between CC (3.85) and STC (3.87). The results suggest that short-stature corn has the potential to be a nutritionally viable forage source for high-producing cows.

Key Words: short-stature corn, organic acid, nutritional quality

Lactation Biology 3: Effects of Dry Period on Mammary Physiology

1592 Mammary gland remodeling during the dry period: Cellular dynamics and their influence on colostrum composition. J. R. Petrou^{*1}, A. J. Fischer-Tlustos¹, K. Klein¹, J. P. Cant¹, M. C. Guenther², J. Laporta², and M. A. Steele¹, ¹*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada*, ²*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI*.

The aim of this study was to investigate the dynamics of cellular proliferation, apoptosis, and connective tissue remodeling in the bovine mammary gland during the dry period and to assess their influence on colostrum composition. Multiparous Holstein cows ($n = 8$) were dried off -58 ± 2.9 d before calving. Cows were fed a controlled energy dry diet (NEL = 1.48 Mcal/kg DM) precalving and a lactating diet (NEL = 1.67 Mcal/kg DM) postcalving. Longitudinal mammary biopsies were performed on d -35, d -21, and d -7 relative to expected calving, at 11.1 ± 5.04 min after colostrum collection (d 0), and at $d 7 \pm 1$ relative to actual calving. Colostrum components were quantified via mid-infrared spectroscopy and IgG via radial immunodiffusion. Tissue samples were fixed, paraffin-embedded, sectioned (5 μ m), and stained with trichrome for connective tissue analysis, or processed for immunohistochemical analysis of cellular proliferation (Ki67) and apoptosis (TUNEL). Connective tissue area, proliferation, and apoptosis were quantified using 4 randomly selected photomicrographs per time point per cow (40 $\times$ magnification). Data were analyzed using PROC GLIMMIX or CORR in SAS, with the fixed effect of time and the random effect of cow. Connective tissue area was higher on d -35 and d -21 than d +7 (34.8% and 35.8% vs. $13.4\% \pm 3.56\%$; $P < 0.01$). Alveolar proliferation peaked on d -7, exceeding all other time points (11.9% vs. $3.5\% \pm 1.37\%$; $P < 0.01$). Stromal proliferation was higher on d -7 compared with d -35, d -21, and d +7 (6.1% vs. 2.2% , 2.4% , and $1.2\% \pm 0.74\%$; $P < 0.01$). Alveolar apoptosis was higher on d -35 vs. all other time points (0.6% vs. $0.2\% \pm 0.04\%$; $P < 0.01$), whereas stromal apoptosis was higher on d -35 compared with d -7 and d 0 (0.4% vs. 0.3% and $0.2\% \pm 0.04\%$; $P < 0.01$). Alveolar apoptosis on d -35 was correlated with colostrum fat ($r = 0.94$; $P = 0.02$), lactose ($r = 0.84$; $P = 0.07$), and IgG ($r = 0.89$; $P = 0.02$) yields, and stromal apoptosis on d -35 tended to be associated with colostrum IgG and fat yield ($r = 0.77$ and $r = 0.82$; $P < 0.09$). These findings suggest that the degree of cellular turnover and tissue remodeling in the mammary gland during the dry period may play a role in colostrum production.

Key Words: colostrum, dry period, involution

1593 Methionine supplementation to heat-stressed transition cows impacts mammary gland cell dynamics and gene expression. B. D. Davidson^{*1}, M. C. Guenther¹, M. Sfulcini², D. N. Sherlock³, D. Luchini³, and J. Laporta¹, ¹*University of Wisconsin–Madison, Madison, WI*, ²*Università Cattolica del Sacro Cuore, Piacenza, Italy*, ³*Adisseo USA Inc., Alpharetta, GA*.

Dry period heat stress (HS) reduces subsequent milk and protein yield. Rumen-protected methionine (RPM) modulates protein metabolism and may alleviate HS effects by enhancing mammary gland (MG) cell dynamics and milk protein gene expression. We evaluated the effect of RPM supplementation on the lactating MG of transition cows under HS. Multiparous Holstein cows were fed a control diet (CN, 2.2% Met of MP) or a CN diet with SmartamineM (MT, 2.6% Met of MP, Adisseo Inc., France) for 6 wk before calving. To induce HS, all MT cows and half of CN cows received an electric heat blanket (EHB) 4 wk before

calving, with the remaining CN being thermoneutral (TN). This resulted in 3 treatments: CNTN, CNHS, and MTHS, which lasted until 28 DIM. Mammary gland biopsies ($n = 8$ per treatment) were performed at 8 and 28 DIM. Tissue was processed and stained with hematoxylin and eosin to visualize MG microstructure (4 images per animal; 20 $\times$) or snap-frozen and stored at -80°C for RNA extraction and gene expression analysis. The transcript abundance of milk protein (*LALBA*, *CSN2*) and 1-carbon metabolism-related (*MAT1A*) genes were measured by RT-qPCR. Data were analyzed using the MIXED procedure of SAS with 2 orthogonal contrasts: CNTN vs. the average of CNHS and MTHS (C1), and CNHS vs. MTHS (C2). Gene expression was analyzed with the $\Delta\Delta\text{CT}$ method (reference: *UTX*, *ACTB*, *KRT8*). At 8 and 28 DIM, HS reduced alveoli number (C1, $P \leq 0.03$; d 8 = 23 vs. 19 ± 1 alveoli; d 28 = 22 vs. 19 ± 1 alveoli) and increased mammary epithelial cell layer area (C1, $P \leq 0.01$; d 8 = $19,802$ vs. $23,361 \pm 1,041 \mu\text{m}^2$; d 28 = $13,050$ vs. $19,299 \pm 1,741 \mu\text{m}^2$). Moreover, at d 8, MT tended to reduce the decline in alveoli number (C2; 17 vs. 20 ± 1 alveoli, $P = 0.10$) and decreased mammary epithelial cell layer area (C2; $25,355$ vs. $21,366 \pm 1,066 \mu\text{m}^2$, $P = 0.01$). At d 8, gene transcript abundance was not different. At d 28, *LALBA* and *CSN2* tended to be upregulated in HS cows (C1; $P = 0.10$), whereas *MAT* was upregulated in CNTN (C1; $P = 0.03$) but downregulated in MTHS (C2, $P = 0.04$). Use of EHB on transition cows induced cellular and molecular alterations in the MG, although RPM supplementation may mitigate some of the negative effects of HS.

Key Words: microanatomy, hyperthermia

1594 Impact of nutrient restriction at dry-off on milk somatic cell composition and transcriptome. L. Cattaneo^{*1}, V. Lopeiato², M. M. Passamonti¹, F. Piccioli Cappelli¹, M. Sfulcini¹, G. Liu³, E. Trevisi¹, and A. Minuti¹, ¹*Department of Animal Sciences, Food and Nutrition, Università Cattolica del Sacro Cuore, Piacenza, Italy*, ²*Department of Veterinary Sciences, Università di Messina, Messina, Italy*, ³*Institute of Zoonosis, College of Veterinary Medicine, Jilin University, Changchun, China*.

Dry-off is a stressful event in dairy cows, particularly if milking is stopped abruptly and milk yield is still high. A safety threshold of 15 kg/d of milk at dry-off has been proposed, and several ways are implemented to achieve this target yield and concurrently promote mammary involution. Nutrient restriction effectively reduced yield but heavily affected metabolism in this phase. This study aimed at assessing whether reducing nutrient density at dry-off would affect milk SCC composition and transcriptome at dry-off. Twenty-six Holstein cows with yield greater than 15 kg/d were either fed only grass hay ad libitum for 7 d before dry-off ($n = 13$) or continued to receive the lactation diet ($n = 13$). After dry-off, both groups received only grass hay for 7 d, and free access to water was always provided. Milk samples were collected at -7, -3, and 0 d relative to dry-off through a portable milking system for RNA sequencing and flow cytometry analysis. Moreover, samples were aseptically collected before dry-off and after next calving for SCC determination and bacteriological culture. Data were analyzed with repeated measures mixed models, and differentially expressed genes were obtained with edgeR considering the effects of time and treatment. Milk yield dramatically decreased during restriction. Milk SCC increased during restriction but was unaltered after calving. No differences in CD3+, CD4+, CD8+, and CD11+ milk cells were detected before dry-off. With nutrient restriction, the expression in milk SCC of 114 genes was downregulated, and that of 14 genes was upregulated, whereas no difference was noted at

–7 d from dry-off. Suppressed pathways were mainly related to the transport of proteins and neurotransmitter receptors, whereas activated pathways were related to immune system activity. These results suggest that severe nutrient restriction at dry-off decreases the activity of the mammary gland and might anticipate the activation of the immune system required for the involution process or recruit different immune cells. Further research is needed to decipher the mechanism underlying these effects and the effects on the next lactation.

Key Words: inflammation, immune system, underfeeding

1595 Heat stress during the dry period reduces feed intake and milk production in the subsequent lactation, but methane emissions intensity is not affected. D. Onan-Martinez^{*1}, M. A. T. de Bari¹, H. Olmo¹, J. Lance¹, J. Magalhães¹, N. Jeronimo¹, J. M. Tricarico², and G. E. Dahl¹, ¹*Department of Animal Sciences, University of Florida, Gainesville, FL*, ²*Dairy Management Inc., Rosemont, IL*.

Multiple studies have documented the negative effects of heat stress (HS) on milk production and feed intake in dairy cattle, including cows that undergo HS during the dry period. The effects of HS on enteric methane (CH₄) production have been evaluated in lactating cows; however, the carry-over effects of HS during the dry period on the subsequent lactation remain unexplored. We hypothesized that decreased milk yield caused by HS in the dry period would increase CH₄ intensity in the subsequent early lactation period. Forty dry cows were enrolled and housed in a freestall barn equipped with individual feed bunks. The cooling treatment (CL = 20) had access to shade, soakers, and fans, whereas the heat-stressed treatment (HS = 20) only had access to shade. After calving, all cows were moved to a neighboring pen also equipped with individual feed bunks, and all were provided with shade, soakers, and fans. At approximately 80 d in milk, a GreenFeed unit was placed in the barn to measure enteric gas output for 28 d in a completely randomized design, and the experiment was conducted in two 28-d time periods with 20 cows each (CL = 10 and HS = 10). Individual feed intake, milk yield, daily CH₄, intensity, and yield were measured during the experiment. Heat stress reduced dry matter intake (DMI; 27.2 ± 0.38 vs. 28.3 ± 0.38 kg/d; *P* < 0.05) and energy-corrected milk (ECM; 49.1 ± 1.07 vs. 55.4 ± 1.10 kg/d; *P* < 0.05). Daily CH₄ emissions tended to be reduced by HS, (320.97 ± 7.07 vs. 339.79 ± 7.4 g/d; *P* = 0.08), whereas yield (CL = 12.38 ± 0.27, HS = 12.08 ± 0.26 g/kg of DMI; *P* > 0.1) and intensity (CL = 6.51 ± 0.19, HS = 6.82 ± 0.18 g/kg of ECM; *P* > 0.1) were not significantly different between treatments. Despite the differences in DMI and ECM, our study suggests that total CH₄ decreases with dry-period HS exposure but yield and intensity are not affected. However, additional data are being collected to confirm or refute that outcome.

Key Words: GreenFeed, enteric methane

1596 Dietary treatment vs. micro-cooling interventions in heat-stressed Italian Holstein heifers. G. Meli, W. Chen, G. Savoini, and G. Invernizzi^{*}, *Department of Veterinary Medicine and Animal Sciences, University of Milan, Lodi, LO, Italy*.

Evidence suggests that heat stress can impair heifers' growth and reproductive performance, potentially compromising their future productive life. The aim of the trial was to evaluate the effectiveness of nutritional strategies and microstructural interventions in enhancing the resilience of heifers under heat-stress conditions. The trial lasted 35 d and was performed during the summer period in northern Italy. Thirty-six Italian Holstein heifers (average age: 10.62 ± 1.01 mo) were grouped in 3 experimental groups (n = 12): F, fed a basal diet with water-cooled mattresses and a micro-cooling system at the level of feeders and waterers; T, fed with the same basal diet supplemented with calcium gluconate at 10 g per heifer per day with no cooling system; C, fed the same basal diet with no cooling system. Temperature, and relative humidity were monitored, and temperature-humidity index (THI) was calculated. Twice a day, at 0800 h and 1400 h, rectal temperature and respiration rate were measured. Feed intake (FI), dry matter intake (DMI), and water intake (WI) were recorded daily. Live body weight (LWB) was recorded once a week, and average daily gain (ADG) and feed conversion ratio (FCR) were assessed. Urine pH was assessed at 0, 17, and 35 d. Data were analyzed using proc. GLIMMIX of SAS 9.4, and significance was set as *P* < 0.05. No significant differences were observed in FI and DMI between the experimental groups. Level of WI was significantly lower for group F during the whole trial (F = 35.47 ± 2.67 vs. C = 44.11 ± 2.67 and T = 47 ± 2.67 L; *P* < 0.05). The F group exhibited higher (*P* < 0.05) LBW compared with T group at d 28 (321.17 ± 3.44 vs. 306.8 ± 3.48 kg) and 35 (327.67 ± 3.44 vs. 317.28 ± 3.42 kg). The ADG was statistically higher (1.13 ± 0.11 vs. 0.74 ± 0.11 kg; *P* < 0.05) and FCR was significantly lower (7.99 ± 1.35 vs. 12.55 ± 1.41; *P* < 0.05) in the F group compared with the T group. Moreover, F animals had the lowest respiration rate (F = 50.02 ± 1.52 vs. C = 56.05 ± 1.52 and T = 56.62 ± 1.52 breaths/min; *P* < 0.05) and rectal temperature when THI exceeded 80 units (F = 38.88 ± 0.06 vs. C = 38.99 ± 0.06 and T = 39.09°C ± 0.06). Overall, heifers with water-cooled mattresses and a micro-cooling system were better able to withstand the summer heat, and calcium gluconate did not counteract negative effects of heat stress; however, no differences were observed between C and T groups.

Key Words: heat stress, heifer, nutritional strategy

Joint Ruminant Nutrition and Animal Health Platform Session: NC2040—The Gut as a Gateway; What We Know, What We Don't, and Where We're Going

1597 Progress in the gut: What we know about “gut health.” G. B. Penner^{1*}, C. A. Bertens¹, and J. R. Aschenbach², ¹*Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada,* ²*School of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany*

“Gut health” is a poorly defined term generally used to indicate adequate functionality of the gastrointestinal tract (GIT) and its associated microbiota. The term “gut health” became gradually popular in the scientific literature only around the beginning of the current millennium and was initially coined regarding humans and monogastric animals. The later advent of gut health studies in ruminants was built on existing research that evaluated aspects of GIT functionality, focusing primarily on ruminal adaptation such as strategies to promote papillae growth to support increased absorptive surface area and adaptation of the ruminal microbiota. Much of this research was performed in dairy calves around weaning and beef and dairy cattle during dietary changes and when fed highly fermentable diets. Collectively, these studies highlighted that increasing diet fermentability generally increased the capacity for short-chain fatty acid (SCFA) absorption with changes in functional activity and enlargement of the epithelial surface. Others evaluated mechanisms for SCFA transport, highlighting that absorption includes SCFA/HCO₃⁻ exchange such that the absorption of SCFA results in the release of HCO₃⁻, helping to stabilize ruminal pH. Likewise, dietary adaptation altered the metabolic pathways involved in SCFA absorption, suggesting increased cholesterol synthesis in addition to ketogenesis. As cattle with ruminal acidosis often had an acute-phase protein response, barrier function properties of the ruminal epithelium were evaluated and partly integrated with studies on the immune system. Later, a focus on hindgut acidosis emerged, leading to the characterization of GIT permeability as an indicator for barrier function and more focused research evaluating GIT immune responses. It is now generally accepted that regions within the small intestine are more permeable than pregastric and large intestinal regions, and that nutritional challenges appear to affect postluminal permeability to a greater extent. Ongoing research efforts are needed to understand factors that promote maintenance of GIT barrier function, and research should expand to include other components of barrier function such as mucus, antimicrobial peptides, and immunoglobulin secretion. Emerging research has focused on the immune-related responses of the GIT to evaluate cell populations, immune pathways induced in response to nutritional challenges, and responses when other organ systems become infected, supporting the concept of the gut-brain axis.

Key words: barrier function, immune response, permeability

1598 Evaluating the effects of fat and colostrum supplementation in milk replacer on gut permeability, hematology, and performance of non-replacement calves. L. Pisoni^{1*}, C. Wang², D. J. Seymour^{1,3}, J. Mergh Leão⁴, A. Keunen⁵, D. L. Renaud², and M. A. Steele¹, ¹*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* ²*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* ³*Trouw Nutrition R&D, Boxmeer, the Netherlands,* ⁴*The Saskatoon Colostrum Company Ltd., Saskatoon, SK, Canada,* ⁵*Mapleview Agri, Palmerston, ON, Canada.*

This study aimed to investigate the impact of fat and colostrum supplementation in milk replacer (MR) on gut permeability, hematology, and

performance in non-replacement calves during the first 28 d after arrival at a calf-rearing facility. At arrival, 48 calves (n = 24 Holstein and n = 24 Holstein × Angus) were allocated to 1 of 3 treatments: (1) low-fat (LF; n = 16) received 6 L of MR (26% CP, 17% fat) from d 1 until d 28; (2) high-fat (HF; n = 16) received 6 L of MR (26% CP, 24% fat) from d 1 until d 21, then transitioned to LF MR until d 28; and (3) extended colostrum (EXT; n = 16) received 6 L of MR (26% CP, 24% fat) supplemented with 140 g of colostrum replacer from d 1 until d 21, then transitioned to LF MR until d 28. All calves were fed MR twice daily and had ad libitum access to water and solid feed. Body weight (BW) and solid feed intake were recorded weekly. Blood samples were taken on d 1, 7, 14, 21, and 28, and gut permeability tests using Cr-EDTA as a marker were performed on d 7, 14, 21, and 28. Data were analyzed using a repeated measure linear regression model in SAS with calf as a random effect and treatment, time, and their interaction as fixed effects. Calf BW, average daily gain, and solid feed intake did not differ ($P = 0.15$, $P = 0.74$, and $P = 0.40$, respectively) between treatments. However, crossbred calves had greater BW ($63.5 \text{ kg} \pm 1.18$ vs. 59.17 ; $P = 0.01$) compared with Holstein calves. White blood cell (WBC) count was greater in HF calves compared with LF calves ($2.21 \times 10^9/\text{L} \pm 0.042$ vs. $2.05 \times 10^9/\text{L} \pm 0.043$; $P = 0.03$), whereas EXT calves showed intermediate values. Gut permeability was not different ($P = 0.26$) between treatments. However, Cr-EDTA concentrations showed a 3.5-fold reduction from wk 1 (866.6 ppb) to wk 28 (245.4 ppb). In conclusion, no differences were observed when supplementing colostrum in MR, whereas MR fat supplementation increased WBC without influencing gut permeability or performance in non-replacement male calves.

Key Words: non-replacement calves, milk replacer fat supplementation, colostrum supplementation

1599 Effects of starch inclusion level in a starter diet for milk-fed beef-on-dairy calves on growth performance and gastrointestinal health. L. Rivas^{1*}, P. A. Lancaster¹, W. E. Brown², R. L. Larson¹, and R. Amachawadi¹, ¹*Beef Cattle Institute, Department of Clinical Sciences, Kansas State University, Manhattan, KS,* ²*Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS.*

Beef-dairy cross calves have twice the liver abscess prevalence as native beef calves, which may be related to early feeding management strategies. The objective of this study was to compare different starch levels in a starter diet on growth performance and gastrointestinal health in beef-dairy cross calves at weaning. Castrated male beef-dairy cross calves (n = 20) were randomly assigned to a high- or low-starch starter grain (44 vs. 24% starch, respectively; n = 10/treatment) fed ad libitum from d 10 of age, and refusals were weighed once daily. Milk replacer was offered twice daily at 3.8 L/d and was increased to 5.7 L/d on d 10 until d 40, then stepped down 1.9 L/d per week until weaning on d 54. Calves were weighed on d 10, 40, 51, 54, and 80. Feces were assigned a categorical score 5×/week, and days of electrolyte therapy were recorded as a measure of scours incidence. Body weight and starter DMI were analyzed with a linear mixed effects model including fixed effects of treatment and day, and random effect of calf. Fecal scores were analyzed with a mixed effects logit model, and days of electrolyte therapy were assessed with a simple linear model. All analyses were done using R v 4.4.1. Prewaning BW increased with time ($P < 0.01$; $52.4 \pm 2.66 \text{ kg}$ on

d 10 vs. 75 ± 2.63 kg on d 54), but there was no evidence of difference between treatments ($P = 0.29$). Starter DMI revealed a significant time $\times$ treatment interaction. There was no evidence of differences in starter DMI on d 40 when milk stepdown began ($P > 0.05$), but low starch had greater starter DMI at weaning on d 54 than high starch ($P < 0.01$; 1.96 vs. 1.65 ± 0.07 kg/d). There was no evidence of differences ($P > 0.05$) between treatments for ADG, fecal score, or days of electrolytes during the preweaning period. There was no evidence of differences between treatments ($P = 0.88$) in postweaning DMI or BW, but postweaning DMI and BW increased with time ($P \leq 0.054$). In conclusion, low starch increased starter DMI during the milk stepdown phase; otherwise starch content of the starter grain had minimal effects on intake, growth, and gastrointestinal health.

Key Words: fecal score, liver abscess, scours

1600 Intestinal integrity and inflammatory markers in neonatal beef $\times$ dairy calves exposed to *Salmonella* infection. K. Migl*, R. Santos, K. M. Holzapfel, S. Gade, L. M. Kluppel, M. Cruz-Penn, and F. Rosa, *School of Veterinary Medicine, Texas Tech University, Amarillo, TX.*

Dairy farmers have been increasing the output of dairy $\times$ beef crosses calves, also known as beef-on-dairy calves. Although beef-on-dairy calves are managed similarly to replacement heifers, whether their diet should be adjusted to improve long-term health and performance remains unclear. In this light, this study aimed to assess the gut health status of beef-on-dairy calves either fed or deprived of colostrum while experiencing enteric infection. Newborn beef $\times$ dairy calves were randomly assigned to either a colostrum-deprived or a colostrum-fed group ($n = 12$ /group), with the latter receiving 2.8 L of pooled colostrum via oral gastric tube within 6 h after birth. Colostrum quality was 26% on the Brix scale. At 8 d of age, a subset of calves in each group were orally inoculated with *Salmonella* Typhimurium. Inoculated calves were euthanized, and intestinal samples were collected at 72 h post-inoculation, whereas non-inoculated calves were euthanized, and intestinal samples were collected at 23 d of age. Ileum samples were subjected to total RNA extraction, gene expression analysis via RT-qPCR, and histological assessments. Data were analyzed using PROC GLIMMIX (SAS 9.4) with group as the fixed effect and calf within group as the random effect. At 72 h post-inoculation, the colostrum-fed inoculated calves had a tendency ($P = 0.07$) for greater *CLDN1* expression relative to the control inoculated calves. However, *CLDN4* did not differ between groups. At 23 d of age, the non-inoculated colostrum-fed group had a tendency ($P = 0.07$) for greater *NFKB* expression compared with the non-inoculated colostrum-deprived calves. In contrast, *TNFA* and *IL10* did not differ between groups. Qualitative histological assessments revealed epithelial necrosis, villi blunting, and absence of goblet cells in the ileum of colostrum-deprived calves relative to colostrum-fed calves at 72 h following *Salmonella* infection. Beef-on-dairy calves deprived of colostrum and under systemic inflammation had epithelial damage. Further investigations are needed to understand the influence of feeding practices on the intestinal health of beef-on-dairy calves.

Key Words: beef $\times$ dairy calves, inflammation, diet

1601 Characterizing ruminal T lymphocytes in lactating dairy cows. L. C. Vandevoorde*, I. G. Moussiaux, and K. C. Krogstad, *College of Food, Agricultural, and Environmental Sciences (CFAES), The Ohio State University, Wooster, OH.*

The rumen is the largest component of the mature bovine digestive tract, but we know little of its immune system and immune functions. Previous research has demonstrated that rumen tissue is heavily populated with immune cells, most being T lymphocytes. We conducted 2 experiments to evaluate ruminal T lymphocyte phenotypes in rumen tissue. The first experiment included 3 ruminally cannulated Holstein dairy cows in an observational experiment. The second experiment included 6 ruminally cannulated cows in a replicated 3×3 Latin square experiment to assess the effect of increasing dietary starch on ruminal immune cell phenotypes. The levels of starch used in experiment 2 were low (20%), medium (25%), and high (30%). In experiment 1, blood and rumen tissue T lymphocyte proportions were analyzed in JMP with a 2-tailed *t*-test. In experiment 2, rumen immune cell phenotypes were analyzed with a mixed model, which included the fixed effect of starch concentration and the random effects of square and cow within square. Rumen evacuations were conducted during each experiment to facilitate rumen papillae harvest. The papillae were then subject to a tissue digestion and cell isolation procedure. The flow cytometry panel included CD45+ (leukocytes), CD3+ (T lymphocytes), CD4+ (helper T cells), and TCRN-24+ ($\gamma\delta$ T cells). The cows in experiment 1 were clinically healthy, consuming 26.0 ± 3.44 kg/d of DMI and producing at 50.6 ± 12.99 kg/d of milk (mean $\pm$ SD). We observed that CD3+ cells constituted $60.8\% \pm 10.10\%$ (mean $\pm$ SEM) of all ruminal CD45+ cells compared with $32.9 \pm 10.10\%$ in blood ($P = 0.01$). The TCRN24+ cells were the most prominent subset of T lymphocytes in rumen tissue and were enriched compared with blood ($36.4\% \pm 5.3\%$ vs. $4.3 \pm 5.3\%$; $P = 0.02$). The CD4+ were not enriched in the rumen compared with blood ($3.5\% \pm 1.2\%$ vs. $6.0 \pm 1.2\%$; $P = 0.18$). We observed a TCRN24+CD4+ population in the rumen that was absent in blood ($17.9\% \pm 3.6\%$ vs. $0.3 \pm 3.9\%$; $P = 0.04$). In experiment 2, starch concentration did not affect T lymphocyte proportions ($P \geq 0.25$), but TCRN24+ were the most prominent subset in rumen tissue. Our data demonstrate that rumen papillae contain $\gamma\delta$ T lymphocytes, which may aid in rumen tissue regulation, maintenance, and function.

Key Words: immunology, rumen, gamma-delta

1602 Optimizing starch concentrations in low-forage diets. I. G. Moussiaux* and K. C. Krogstad, *The Ohio State University, Wooster, OH.*

This abstract is part of the ADSA-Graduate Student Competition: Production (MS Oral Semifinalists).

Forage quality and availability will be challenged due to fluctuating weather patterns. Optimizing low-forage diets may enhance farm resilience when forage is limited. Our objective was to determine the optimal dietary starch concentration in low-forage diets. We hypothesized that increasing dietary starch in a low-forage diet (12.5% forage NDF [fNDF]) will increase milk yield, reduce milk fat yield, and increase milk fat depression related milk fatty acids. Starch was increased by adding more corn grain and reducing soybean hulls. Six multiparous Holstein cows (29–92 DIM) were assigned to treatments containing (1) 20% starch (LS), (2) 25% starch (MS), or (3) 30% starch (HS) in a replicated 3×3 Latin square design, with 21-d periods. The diet compositions were 34, 30, and 27% NDF for LS, MS, and HS respectively. The CP was 18% for each diet. Statistical models included random effects of square and cow within squares. We assessed the linear (L) and quadratic (Q) effects of dietary starch concentrations. Rumen pH and fecal pH were unaffected (5.84, 5.78, 5.87; L, $P = 0.48$; Q, $P = 0.43$). Plasma Hp, an inflammatory biomarker, was also not affected by starch (L, $P = 0.93$;

Q, $P = 0.14$). Starch did not affect DMI (37.12, 35.72, 36.40 kg/d; L, $P = 0.33$; Q, $P = 0.12$) or milk yield (48.9, 47.4, 49.9 kg/d; L, $P = 0.53$; Q, $P = 0.16$). Increasing starch linearly reduced milk fat percentage (3.98, 3.72, 3.34%; L, $P = 0.04$; Q, $P = 0.25$) and tended to quadratically reduce milk fat yield (1.91, 1.55, 1.63 kg/d; L, $P = 0.05$; Q, $P = 0.08$). Short-chain fatty acids C6:0, C8:0, C10:0 (L, $P \leq 0.03$; Q, $P \leq 0.31$) and *trans*-10,*cis*-12, C18:2 (0.006, 0.009, 0.03%; L, $P < 0.01$; Q, $P = 0.45$) were significantly affected. Milk protein percentage tended to linearly decrease with increasing starch (3.45, 3.35, 3.24%; L, $P = 0.07$; Q, $P = 0.87$), whereas milk protein yield remained unchanged (1.67, 1.58, 1.60 kg/d; L, $P = 0.16$; Q, $P = 0.15$). Increasing starch quadratically reduced ECM (52.84, 46.98, 48.95 kg/d; L, $P = 0.09$; Q, $P = 0.05$). Reduced starch concentration increased milk fat yield and energy-corrected milk yield in low-forage NDF rations.

Key Words: starch, digestibility, low-forage diet

1603 Assessing an ex vivo assay with gastrointestinal tissue sections to investigate mucosal immune responses in dairy calves. P. O. McDonald*, L. K. Mamedova, and B. J. Bradford, *Michigan State University, East Lansing, MI.*

Scours remain a leading cause of calf morbidity and mortality, leading to steep economic losses for producers each year. Mucosal immunity along the gastrointestinal tract is therefore essential for maintaining calf health. Our objective was to evaluate whether challenging intestinal tissue explants ex vivo would lead to quantifiable immune responses. Sections (25 mm²) of ileum and mid-jejunum from male dairy calves (n = 3; 8–10 d old) were collected at harvest and placed mucosal side up in a 24-well plate containing biopsy pads and prewarmed medium. To simulate scours, explants were stimulated with either control (medium only), bovine rotavirus (TCID₅₀, $\sim 1.8 \times 10^7$ per well) or *Escherichia coli* (1×10^7 per well; field isolate). The plate was incubated at 37°C with 5% CO₂ for 2 h. Explants were then placed in Trizol for RNA isolation and medium collected for cytokine analysis. Transcript abundance was determined by qPCR for *TNF* and *IL6* relative to *RPS9* (reference gene). Supernatants were analyzed with a bovine multiplex ELISA for cytokines and chemokines. Data were analyzed (SAS GLIMMIX) with the fixed effects of stimulant, tissue location, and their interaction and the random effect of calf. Pathogenic stimulation increased mRNA abundance ($P < 0.05$) of *TNF* (undetected in 7/18 samples) and *IL6* (undetected in 2/18 samples) above control, but was not significantly affected by pathogens, tissue locations, or their interaction (all $P > 0.40$). Both tissue segments had detectable net release ($P < 0.05$) of the following proteins: IL1b, IL6, IL10, MIP1a, MCP1, MIP1b, and VEGFa. Only ileal explants had measurable net release of IFN γ , IL1a, IL17a, IL36a, and TNF. Ileal segments had or tended to have greater release of IFN γ ($P = 0.03$), IL1a ($P = 0.04$), IL6 ($P = 0.08$), IL10 ($P = 0.07$), IL17a ($P = 0.03$), IL36Ra ($P = 0.01$), MIP1b ($P = 0.049$), TNF ($P = 0.06$), and VEGFa ($P < 0.01$). Despite this evidence of immune activity in the explants, main effects of stimulant and the interaction with tissue location were not significant for any cytokine. These results demonstrate that intestinal explants, particularly from the ileum, generate measurable secretion of cytokines. Responses to bacterial and viral enteric pathogens were not detected in this small pilot study.

Key Words: explant, mucosal immunology, scours

1604 Effects of a synbiotic on gastrointestinal permeability and performance in peak milk cows during feed restriction. V. L. Daley*, C. M. K. Bradley, T. L. Crews, and O. N. Genther-Schroeder, *Purina Animal Nutrition, Gray Summit, MO.*

The objective of this study was to evaluate the effects of a synbiotic on total-tract gastrointestinal permeability (TTP), animal health, and performance in peak-lactation cows using a feed restriction (FR) model. Multiparous Holstein cows (n = 24), housed in a freestall barn with Calan gates, were assigned a treatment (trt) based on previous 305-d milk yield (MY) and expected calving date in a completely randomized design. Treatments consisted of a control (CON) or a synbiotic group (SYN, 14 g/d *Bacillus subtilis* spores and yeast products on a DM basis, Amulet) mixed into TMR and fed from 28 d before calving to the end of the FR challenge (89 $\pm$ 9 d postpartum). For the FR challenge, all cows (74 $\pm$ 9 DIM, 51 $\pm$ 10 kg/d MY) were moved to tiestalls, and fed and milked 3 $\times$ /d for 14 d. The study included an ad libitum period (AL, d 1 to 5), followed by a FR period (FR, d 6 to 9) targeting 50% of the average daily DMI from the AL period, and a refeeding period (RE, d 10 to d 14) targeting 110% of AL DMI. The TTP was measured during the AL and FR periods (d 3 and 6) by comparing plasma Cr recovery from cows orally dosed with 1.5 L of 180 mM Cr-EDTA. All cows were fitted with a jugular catheter on d 2 for blood collections that were taken daily from d 2 to 13 for metabolites and inflammatory markers, and at 0, 2, 4, 8, 12, 18, and 24 h post-dosing of Cr-EDTA to estimate the area under the curve (AUC). Two mixed models were fitted to the data to include fixed effects of trt, time or period, their interactions, and the random effect of cow within trt. No negative health issues were detected during the FR challenge. Both DMI and ECM were similar between trt ($P > 0.05$). We found that FR reduced MY ($P < 0.01$, 18% decrease) and increased serum NEFA ($P < 0.01$, 70% increase) and BHB ($P < 0.01$, 30% increase). Further, FR increased TTP compared with AL (AUC: $P < 0.01$, 16% increase) but was not affected by trt ($P > 0.05$). Serum haptoglobin was similar between groups ($P > 0.05$). Cows fed SYN tended to have lower milk SCS ($P = 0.09$) than CON. Overall, SYN may prevent milk SCS increase during stress in peak milk cows.

Key Words: synbiotic, intestinal permeability, somatic cells

1605 The effects of an intramammary LPS challenge in early-lactation cows fed a direct-fed microbial on performance, systemic inflammation, and gastrointestinal tract permeability. C. Bertens*,¹ D. Paulus-Compant,² C. Stoffel,² N. Hogan,³ A. Facciolo,⁴ and G. B. Penner,¹ ¹University of Saskatchewan, Saskatoon, SK, Canada, ²Papillon Agriculture Services, Easton, MD, ³Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada, ⁴Vaccine and Infectious Disease Organization (VIDO), University of Saskatchewan, Saskatoon, SK, Canada.

This study evaluated the effects of a direct-fed microbial (DFM) on DMI, milk yield, systemic inflammation, and regional gastrointestinal tract (GIT) permeability following an intramammary lipopolysaccharide (IMM-LPS) challenge. Lactating Holstein cows (n = 34, 56 d in milk [DIM]) with somatic cell count (SCC) <250,000 cells/mL were used, including 15 that were ruminally cannulated. Cows received either 28 g/d of DFM (a blend of probiotics, postbiotics, and exogenous enzymes; DFM) or no DFM (PLB) for 26 d before being challenged with nothing (CON) or IMM-LPS (200 μ g of *Escherichia coli* O111:B4 LPS; LPS) on d 27 into both rear quarters (DFM-LPS, n = 8; DFM-CON, n = 9; PLB-LPS, n = 8; PLB-CON, n = 9). Post-challenge observation included 3 phases (d 1 to 5 [CHAL], d 6 to 10 [REC1], and d 11 to 15 [REC2]) to assess DMI, milk yield, regional GIT permeability (using a bolus dose of Cr-EDTA into the rumen and Co-EDTA into the abomasum followed by blood sampling for calculation of area under the curve [AUC]), and blood markers. The effects of DFM, LPS, time, and the 2- and 3-way interactions were tested using GLIMMIX. We found that DMI decreased by 30%, 11%, and 9%, and milk yield decreased 50%,

24%, and 9% from d 1 to 3 for LPS relative to CON (LPS $\times$ day, $P < 0.01$). The DMI decreased by 12%, 3%, and 2% in CHAL, REC1, and REC2, respectively, for LPS compared with CON (LPS $\times$ phase, $P < 0.01$). The LPS cows had 18% less milk in CHAL, 3% less in REC1, and 1% less in REC2 than CON (LPS $\times$ phase, $P < 0.01$). Exposure to IMM-LPS increased rectal temperature over CON by 2.8°C 6 h post-challenge (LPS $\times$ hour, $P < 0.01$). Plasma haptoglobin was increased by 642-, 41-, and 5-fold ($P < 0.01$), on d 1, 7, and 12 for LPS over CON, and milk SCC increased by 1,868-, 11-, and 2-fold ($P < 0.01$) for LPS relative to CON in CHAL, REC1, and REC2. On d 1, the Cr and Co AUC were 24% (LPS $\times$ phase, $P < 0.01$) and 28% (LPS $\times$ phase, $P < 0.01$) lower for LPS than CON, respectively. On d 6 the Co AUC was 33% lower for LPS than CON, but Cr AUC did not differ on d 6 or 11, and Co AUC did not differ on d 11. The IMM-LPS challenge induced local and systemic inflammation that persisted longer than reduced DMI and milk yield. Total and post-ruminal GIT permeability may transiently decrease in response to IMM-LPS, but the DFM used may not attenuate the response.

Key Words: probiotic, inflammation, mastitis

1606 Gut health in ruminants: Where to from here? A. H. Laarman^{*1}, K. C. Krogstad², and J. S. Osorio³, ¹*University of Alberta, Edmonton, AB, Canada*, ²*The Ohio State University, Wooster, OH*, ³*Virginia Polytechnic, Blacksburg, VA*.

Supporting high-yield dairy production requires energy-dense diets that, because of their rapid fermentability, create an acidotic pressure,

especially in the rumen. Subacute ruminal acidosis, in particular, has long been linked to compromised fiber digestibility, reduced ruminal barrier integrity, and lower productivity in dairy cattle. In past decades, much research has focused on minimizing lipopolysaccharide release and mitigating rumen pH depression. In so doing, the hindgut also becomes at risk, as energy-dense diets can increase post-ruminal starch flow, causing excessive hindgut fermentation and disrupting barrier function in the hindgut. Consequently, rumen acidosis itself has come to be seen as a more holistic complex involving the entire gastrointestinal tract, whereby rumen pH, hindgut pH, and immune response all play roles in the adverse productivity effects seen in dairy cattle. Seen as a complex disorder that is more than just depressed rumen pH, ruminal acidosis requires a more holistic response in our diet formulations and nutritional programs. We must improve our understanding of how nutrition, management, and stress influence rumen tissue structure, hindgut barrier function, immunophenotype, and inflammatory tone. For instance, recent research demonstrated that the immunophenotype of the rumen wall is a tolerant and resilient structure that exhibits a differential response to lipopolysaccharides from different microbial species, highlighting the need for improved understanding of not only the microbial response but also the cow response and their interaction. Going forward, the microbe-immune interface is likely to play an important role in tailoring diets for high-producing dairy cows, enhancing rumen fermentation and buffering in the rumen while supporting the immunological responses of the cow.

Key Words: ruminal acidosis, hindgut acidosis, immune response

Joint Reproduction and Physiology and Endocrinology Symposium: Physiological Insights into Ovarian Follicle Development— Laying the Foundation for Enhanced Fertility

1607 The role of the preovulatory follicle in oocyte metabolism and competence for embryo development in cattle. S. E. Moorey*, *Department of Animal Science, University of Tennessee, Knoxville, TN.*

Oocyte competence for embryo development is greatly influenced by its surrounding microenvironment during oocyte maturation. The physiologically mature preovulatory follicle is the normal, and presumably ideal, microenvironment of the maturing oocyte. Our overarching hypothesis is that the intrafollicular milieu of physiologically mature preovulatory follicles provides the necessary microenvironment to metabolically program oocytes for successful embryo development. Studies by our team have demonstrated that the follicular fluid metabolite milieu differs between preovulatory follicles of greater and lesser maturity and that such differences have direct effects on the oocyte. Numerous follicular fluid metabolites involved in glucose and amino acid metabolism were more abundant in preovulatory follicles of greater versus lesser physiological maturity. Additionally, abundance of transcripts encoding glycolytic enzymes was higher in cumulus cells from follicles of greater maturity, and both ATP and levels of key transcripts in the mitochondrial respiratory chain were higher in oocytes from preovulatory follicles of greater versus lesser maturity. We supplemented *in vitro* maturing oocytes with follicular fluid from preovulatory follicles of greater or lesser maturity to determine causality of differing follicular fluid milieu on cumulus-oocyte metabolism and oocyte competence for early embryo development. Follicle maturity status had robust effects on cumulus-oocyte complex consumption or production of key metabolites in glucose, purine, and amino acid metabolism pathways during oocyte maturation. We also observed hastened development rate in embryos arising from *in vitro*-matured oocytes supplemented with lesser-maturity follicular fluid. Furthermore, RNA sequencing of resulting blastocysts suggested more efficient metabolism and Wnt/ β -catenin signaling in blastocysts from oocytes matured in preovulatory follicular fluid from greater-maturity follicles. In contrast, transcriptome profiles of blastocysts from oocytes matured in preovulatory follicular fluid from lesser-maturity follicles indicated oxidative stress and dysregulated cell division. These recent findings by our laboratory indicate effects of follicle maturity, and the resulting follicular fluid microenvironment, on the maturing oocyte, which persist throughout early embryo development and hold promise for improving reproductive efficiency in cattle.

Key Words: follicle-oocyte metabolism, oocyte competence, preovulatory follicle

1608 Effect of subclinical ketosis on ovarian functions in dairy cows. N. Neeraj*, N. Sun, and R. Duggavathi, *Department of Animal Science, McGill University, Montreal, QC, Canada.*

Subclinical ketosis (SCK) is a major metabolic disorder in dairy cows. Multiple large-scale studies have reported an association between SCK during early lactation (1–6 wk after calving) and poor reproductive performance during the subsequent breeding period (9–15 wk post-calving). However, the mechanisms of how SCK influences fertility, specifically ovarian functions, are not fully understood. The objective of this study was to evaluate the association between SCK and ovarian functions. We monitored 50 lactating Holstein cows (11 primiparous

and 39 multiparous) for SCK during the first 42 d in milk (DIM) using thrice-weekly blood sampling and cow-side blood BHBA testing. The cows were grouped as Healthy ($n = 25$ cows) with blood BHBA <1.2 mmol/L during 5–42 DIM. The SCK cows (BHBA 1.2–2.9 mmol/L) were grouped as Early SCK ($n = 10$ cows) and Late SCK ($n = 15$ cows) if a positive ketosis test occurred during 5–15 and 16–42 DIM, respectively. From 43 DIM onward, daily ultrasonographic examination of the ovarian dynamics and blood sampling were performed for 1 interovulatory period. All statistical analyses were performed using SAS 9.4 (SAS Institute Inc.). Follicular and luteal data were analyzed using one-way ANOVA, PROC MIXED was used to analyze serum glucose and progesterone, and differences between means were evaluated using Tukey's multiple comparison test. Serum glucose concentrations during the first 42 DIM revealed that Healthy cows had greater ($P < 0.0001$) mean glucose concentration (2.9 ± 0.1 mmol/L) than Early and Late SCK cows (2.3 ± 0.2 and 2.6 ± 0.1 mmol/L, respectively). Examination of the ultrasound data revealed that the number of follicles at the emergence of the first follicular wave was greater ($P < 0.001$) in Healthy cows (14.1 ± 0.4) than in Early and Late SCK cows (11 ± 0.5 and 11.7 ± 0.5 , respectively). Although the mean largest corpus luteum diameter and peak progesterone concentrations were similar among cow groups, Healthy cows had higher mean serum progesterone concentrations during the first 15 d of the estrus cycle than Early and Late SCK cows ($P < 0.05$). These data demonstrate a negative association between SCK within 42 DIM and ovarian (follicular and luteal) functions during the subsequent estrous cycle. This is the first study reporting the apparent knock-on effects of SCK on ovarian functions. Hence, frequent monitoring of dairy herds during the first 6 wk of lactation is necessary to optimize reproductive performance, resulting in sustainable dairy production.

Key Words: dairy cow, ovarian activity, subclinical ketosis

1609 Antral follicle count and dominant follicle size in dairy cattle. K. J. Alward^{*1}, A. B. Pollock², and A. D. Ealy², ¹*West Texas A&M University, Canyon, TX,* ²*Virginia Polytechnic Institute and State University, Blacksburg, VA.*

Antral follicle count (AFC) in cattle has been widely used as a marker of response to superovulation protocols and has been positively linked with embryo production *in vitro*. Dominant follicle (DF) size at the time of ovulation has also been linked to fertility, with cows that ovulate smaller follicles having reduced conception rates. We hypothesized that DF size and follicular fluid (FF) volume are reduced for low-AFC cattle, which may explain differences in fertility and embryo viability. The AFC for a herd of nonsynchronized lactating Holstein cattle ($n = 85$) was determined via transrectal ultrasonography (TRUS) by a single operator. Cows in the top 25% (30 ± 2 follicles) were classified as high AFC, and cows in the bottom 25% (13 ± 0 follicles) were classified as low AFC. High-AFC ($n = 5$) and low-AFC ($n = 6$) cows were balanced by parity and subjected to a presynchronization protocol (GnRH d 0, PGF_{2a} d 7, GnRH d 10) followed by a synchronization protocol (GnRH d 17, PGF_{2a} d 24 and 25, GnRH 32 h later) and DF measurement and FF collection 16 h later on d 27. Cows were subjected to a second synchronization, which began on d 37 (GnRH d 37, PGF_{2a} d 44 and 45, GnRH 32 h later) and DF measurement and FF collection 16 h later on d 47. Follicle size was determined via TRUS, and collection of DF

FF was completed via ultrasound-guided transvaginal aspiration. All cows received an epidural nerve block with lidocaine. The perineum was sanitized, and an ultrasound-guided transvaginal aspiration device equipped with a 21-gauge spinal needle and sterile plastic tubing for FF collection was inserted into the vagina. The needle was advanced through the vaginal wall into the DF antrum, and fluid was aspirated into a 5-mL syringe and measured. Data were analyzed with PROC GLM of SAS 9.4 for follicle area and FF volume, with AFC classification as the main effect and collection time as the repeated measure. Recovery of DF FF was 100% for both groups. Neither follicle area (low = $358 \pm 69 \text{ mm}^2$; high = $235 \pm 35 \text{ mm}^2$) nor FF volume (low = $2.88 \pm 0.66 \text{ mL}$; high = $2.52 \pm 0.61 \text{ mL}$) differed by AFC classification ($P = 0.15$; $P = 0.70$). We expected to find lower DF size in low-AFC cows as a linkage to reduced fertility outcomes in these animals; however, this indicates that other factors influence fertility outcomes in low-AFC cattle beyond DF size. Future research will increase animal numbers and evaluate hormone concentrations of FF to investigate differences in fertility.

Key Words: dairy, follicle, antral follicle count (AFC)

1610 Risk factors for anovulation in Holstein cows. L. Campora*, E. Morrison, E. Ribeiro, R. Couto Serrenho, and S. J. LeBlanc, *University of Guelph, Guelph, ON, Canada.*

This longitudinal study investigated cow-level risk factors for anovulation to the start of the breeding period. Holstein cows ($n = 2,975$; 114 ± 8 cows/herd) from 26 herds in Ontario using automated activity monitors as a primary tool for insemination were enrolled. Body condition score (5-point scale) change (ΔBCS) between wk 1 and 8 was categorized into ≤ -0.5 , -0.25 , or ≥ 0 . Metritis was assessed weekly by researchers. Purulent vaginal discharge (PVD; $>50\%$ pus) was diagnosed at wk 4, 6, and 8, and endometritis (ENDO; $>5\%$ PMN in uterine cytology) was diagnosed at wk 4, forming a 4-level variable based on combinations of PVD at any time and ENDO. Lameness (LAME) was identified in cows with locomotion score $\geq 3/5$ at wk 8. Serum progesterone $<1 \text{ ng/mL}$ at all of wk 4, 6, and 8 identified anovular (ANOV) cows. A multivariable logistic regression model of ANOV with the explanatory variables above as fixed effects and herd as a random effect was built in R, retaining variables with $P \leq 0.05$. Reporting LSM (95% CI), we found that 12% (10%–14%) of cows were ANOV to wk 8. Parity, uterine health, ΔBCS , and lameness were associated with ANOV. Younger cows were more likely to be ANOV using parity 2 as the referent: parity 1 (14.8%; 12%–18.1%), parity 2 (9.6% (7.4%–12.5%); parity 3 (10.8%; 8.7%–13.3%). Metritis was not associated with ANOV. Endometritis, but not PVD alone, was associated with greater prevalence of ANOV than in healthy cows: neither PVD nor ENDO (44% of cows) 9% ANOV (8%–12%); PVD only (16% of cows) 11% ANOV (8%–15%); ENDO only (19% of cows) 14% ANOV (11%–18%); both PVD and ENDO (21% of cows) 16% ANOV (12%–20%). Accounting for the other variables, cows with $\Delta\text{BCS} \leq -0.5$ (43% of cows) were more likely to be ANOV (13%; 11%–16%) than cows with $\Delta\text{BCS} -0.25$ (37% of cows) (10%; 8%–13%); there was no difference in the 20% of cows with $\Delta\text{BCS} \geq 0$ (11%; 9%–15%) versus $\Delta\text{BCS} -0.25$. Lame cows at wk 8 (6% of cows) were more likely to be ANOV (22%; 15%–24%) than non-lame (11%; 9%–13%). These findings highlight several factors associated with ANOV. Considering ΔBCS without health variables or PVD without ENDO may bias their associations with ANOV.

Key Words: endometritis, lameness, cyclicity

1611 Effect of dosage, and interval between equine chorionic gonadotropin administration and ovum pickup on the follicular

population, recovery, quality, and in vitro developmental competence of oocytes in Sahiwal cattle. M. A. Afzal^{1,2}, J. Ali², M. Saleem³, M. Nawaz⁴, and A. Riaz², ¹University of Missouri, Columbia, MO, ²University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan, ³Islamia University of Bahawalpur, Bahawalpur, Punjab, Pakistan, ⁴Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Punjab, Pakistan.

This study aimed to optimize the dose of equine chorionic gonadotropin (eCG) and interval between eCG administration and ovum pickup (OPU), and to evaluate the carryover effect of a single eCG dose on subsequent OPU in terms of follicular population, recovery, quality, and in vitro developmental competence of oocytes in Sahiwal cattle. In experiment 1, cattle ($n = 28$) were divided into 4 groups ($n = 7$): 0 IU eCG (control), 250 IU eCG, 500 IU eCG, and 750 IU eCG. In experiment 2, cattle ($n = 18$) received 500 IU eCG and were assigned to 3 groups ($n = 6$) based on the interval between eCG injection and OPU: 48 h, 60 h, and 72 h. In experiment 3, cattle ($n = 6$) received 500 IU eCG and underwent 3 OPU every 60 h to assess carryover effect. All cattle were presynchronized with 2 doses of 0.150 mg D-cloprostenol 11 d apart. At 48 h after the second dose, dominant follicle ablation was performed, along with insertion of an intravaginal progesterone device (CIDR). Dosage of eCG was administered 2 d later. On the day of OPU, CIDR were removed and follicles aspirated using ultrasound-guided transvaginal OPU technique. Data were analyzed using SAS proc. GLIMMIX. In experiment 1, medium-sized follicle numbers and oocyte recovery were higher ($P < 0.05$) in 500 IU (2.57 ± 0.31 ; 7.28 ± 0.50) and 750 IU (2.28 ± 0.30 ; 6.00 ± 0.51) groups than in 250 IU (1.28 ± 0.31 ; 5.00 ± 0.52) and control (0.57 ± 0.29 ; 4.85 ± 0.52) groups. However, 500 IU was optimal ($P < 0.05$), as 750 IU did not improve oocyte quality ($P > 0.05$). In experiment 2, the proportion of medium follicles was higher in the 60-h (32.00 ± 0.72) and 72-h (30.00 ± 0.64) groups ($P < 0.05$) than in the 48-h group (14.04 ± 0.58). Grade AB and ABC oocytes were higher in the 60-h group (47.61 ± 1.75 ; 76.19 ± 2.05) than in the 48-h (26.08 ± 1.16 ; 52.17 ± 1.58) and 72-h (25.00 ± 0.78 ; 50.00 ± 1.20) groups ($P < 0.05$). However, in vitro developmental competence was unaffected ($P > 0.05$) by the interval between eCG and OPU. In experiment 3, a sharp decline ($P < 0.05$) in follicular population and oocyte quality was observed after the first OPU, indicating no carryover effect of eCG. In conclusion, 500 IU eCG with a 60-h interval before OPU was most effective for ovarian stimulation in Sahiwal cattle, but a single dose was insufficient for repeated OPU.

Key Words: ovum pickup (OPU), equine chorionic gonadotropin (eCG), Sahiwal cattle

1612 Novel mechanisms conferring resistance to regression during luteal development and pregnancy. C. H. K. Hughes*, *Department of Animal Science, Penn State University, University Park, PA.*

Fertility in the dairy cow is dependent on normal function of the corpus luteum (CL). The CL is a transient endocrine gland that forms following ovulation and produces the progesterone essential to pregnancy maintenance. In the absence of a pregnancy, the CL regresses in response to uterine prostaglandin ($\text{PGF}_{2\alpha}$), allowing for another ovulation. Interestingly, before d 5 of the bovine estrous cycle, the CL fails to regress in response to $\text{PGF}_{2\alpha}$, whereas after d 5, CL regress when exposed to $\text{PGF}_{2\alpha}$. To evaluate global physiological changes in CL of differing capacity to regress in response to $\text{PGF}_{2\alpha}$, we compared the transcriptome and proteome of CL collected on d 4 and 6 of the estrous cycle. We identified proteins associated with apoptosis and cell death that increased on d 6, whereas a miRNA that targets these proteins,

miR-125b-5p, was increased on d 4. Luteal cells were transfected with this miRNA, resulting in resistance to cytokine-induced cell death in culture. In summary, changes in protein expression and alteration of miRNA-mediated repression between d 4 and 6 may make the CL more susceptible to PGF_{2α}-induced cell death. Similarly to changes observed in developing CL, in early pregnancy, there are mechanisms of altered luteal PGF_{2α} responsiveness, but the regulation of these mechanisms is poorly understood. Interferon tau (IFNT) is the primary maternal recognition of pregnancy signal in the cow. During the time of IFNT secretion, the CL of pregnancy is transiently more resistant to the actions of PGF_{2α} than the CL of the cycle. To evaluate the effects of IFNT on luteal function, we infused IFNT into uteri of nonbred heifers 2× per day for 3 d (d 14–17 of the cycle). The CL were collected and RNaseq

was performed. Relative to controls, IFNT resulted in changes to mRNA involved in interferon signaling and antiviral response. Interestingly, among these genes were 3 poly(ADP-ribose) polymerase (PARP), PARP9, 12, and 14, which promote DNA damage response and cell survival. In summary, these studies have identified novel candidate genes that could be targeted to develop technology to support fertility in dairy cattle. This project was supported by USDA Agriculture and Food Research Initiative Predoctoral Fellowship no. 2017-67011-26062 to CHKH, Competitive Grant no. 2012-67015-30212 to JLP, and Penn State Department of Animal Science startup funds to CHKH.

Key Words: corpus luteum, prostaglandin F_{2α}, luteolysis

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1613 Enhancing the usability of large language models in dairy science through an agentic system. E. Liu^{*1}, H. Yang¹, M. van Leerdam¹, M. J. VandeHaar², and M. Hostens¹, ¹*Cornell University, Ithaca, NY*, ²*Michigan State University, East Lansing, MI*.

Large language models (LLM) have potential to revolutionize dairy science, but without proper design, they can hallucinate and generate irrelevant or even factually incorrect responses. Additionally, existing LLM and their tools are not well-adapted to dairy science, limiting their practical applications. To address these challenges, we developed a 2-component agentic system: (1) a decision-support chatbot leveraging the *Journal of Dairy Science* (JDS) for accurate insights, and (2) a natural language interface for interacting with statistical models and visualizing results in real time. The chatbot integrated Llama70B as both the decision node and chat model, with Databricks-bge-en serving as the embedding model. All publicly available JDS abstracts and their metadata since 1917 were compiled through the PubMed API and stored in FAISS for efficient vector search. A retrieval-augmented generation approach, known as RAG, ensured responses were grounded in JDS literature, with the top 5 sources cited for each user query. To deal with queries beyond the scope of JDS, a web-search agent was created and safeguarded by an auto-grader agent that was backed by Gemini2.0 and verified information to prevent hallucination. The second component of the agentic system connected Llama70B with a lactation curve model, enabling users to interact with this milk yield prediction model via natural language and visualize key factors influencing outputs. This is the first chatbot prototype designed to provide dairy-specific insights to scholars, consultants, and farmers, and demonstrates the feasibility of integrating LLM with domain-specific models for broader adoption. Such an interface is particularly valuable in developing regions with limited statistical education and technical support. However, challenges remain. Llama70B is too large for easy deployment on personal devices, and the toolset requires further expansion. Future work will focus on developing a lightweight LLM suitable for lower-power farm computers and integrating additional statistical and machine learning models to enhance usability and impact.

Key Words: artificial intelligence, large language model, retrieval-augmented generation

1614 Modeling a path forward for expanding US dairy producers. L. Olthof^{*1}, K. Dhuyvetter², C. Clark³, and B. J. Bradford¹, ¹*Department of Animal Science, Michigan State University, East Lansing, MI*, ²*Animal Health Division, Elanco, Greenfield, IN*, ³*MSU Extension, Michigan State University, East Lansing, MI*.

The US dairy industry continues to consolidate rapidly. This shift has largely been due to advantages in economies of scale for large farms, and for many small dairies, increasing scale is the most feasible path to financial sustainability. To determine what dairy farm expansion strategy is most likely to improve farm viability, 4 common scenarios were evaluated, estimating probabilities of financial success while considering dairy herd metrics, crop and nutrient management constraints, and environmental and economic implications. Monte Carlo simulations with over 70 parameters varied (feed costs, milk price, interest rate, etc.) were used to assess return on investment, profitability, and cash flow ranges for the following business expansion strategies for a 250-cow dairy: (A) doubling land, cattle, and facility capacity; (B) increasing cattle numbers and facility capacity more drastically without purchasing

crop land, instead relying on more purchased forages; (C) increasing cattle numbers and facility capacity more drastically without additional land by feeding a diet heavier in commodities and byproducts; or (D) expanding modestly while reducing labor costs through automated milking system investment. Strategies were compared using a uniform capital investment of \$4.8 million. Scenario B had the greatest mean return on investment after 10 yr ($103\% \pm 68.4\%$) and annual net profit ($\$497,429 \pm \$999,186/\text{yr}$) with the least debt-to-asset ratio ($27.6\% \pm 2.6\%$). Scenario D had the least labor cost ($\$0.67/\text{kg}$ milk) and the least variable profit. Sensitivity analyses showed that scenario B remained the most profitable option despite changes in interest rates, cow value, loan repayment rates, feed price decreases, and milk price increases. Scenario D became the most profitable scenario if robotic milking increased milk production by 22% relative to conventional milking, if milk price reduced 8%, or if feed cost increased 14%. In summary, devoting capital to increase herd size rather than acquiring land was the most profitable strategy, on average; however, farm-specific conditions affect this finding. Therefore, an online simulation tool is now available to assess farm-specific scenarios.

Key Words: expansion, modeling, dairy robotics

1615 Comparative analysis of cropping strategies on income over feed cost (IOFC) in dairy farming using a virtual farm model. J. C. Becker^{*}, H. D. Karsten, T. S. Adams, and L. A. Holden, *The Pennsylvania State University, State College, PA*.

Cropping strategies can differ in their environmental implications, which can significantly influence the economic outcomes of dairy farming, particularly through feed costs. The objective of this study was to compare the effects of 3 different cropping treatments on income over feed cost (IOFC) using a virtual farm model for 3 simulated herds of 65 milking cows each. All cropping systems were no-till and grew corn silage and alfalfa. A business-as-usual (BAU) treatment used surface broadcast manure, applied standard herbicides and insecticides on 48.5 ha, and purchased other feeds. Two other crop systems used integrated pest management for insects on 97 ha, enabling them to also grow corn grain, soybeans, and winter and summer annual forages. The broadcast manure standard herbicide treatment (BMSTD) used broadcast manure and standard herbicide applications; and the final treatment used injected manure with integrated weed management (IMIWM). Over 5 years, all rotations were managed and harvested in research plots, and forage and feed quality data were used in the virtual farm model for lactating cows. Rations were formulated using NASEM, and IOFC was calculated based on actual crop production costs. One-way ANOVA were run in R to determine whether the financial measures were different among treatments or years. Significance was declared at $P < 0.05$. Results indicated that there were no significant differences among cropping treatments in net energy (NE) IOFC ($P = 0.34$) or metabolize protein (MP) IOFC ($P = 0.72$.) Both NE IOFC and MP IOFC differed significantly with year ($P < 0.001$). This study demonstrates that dairies can employ environmentally sound cropping practices while maintaining IOFC. Also, IOFC was highly influenced by yearly variations, driven by fluctuations in feed costs and milk prices. Cropping strategies had less influence on IOFC than yearly fluctuations in milk prices.

Key Words: dairy sustainability, income over feed cost (IOFC), feed cost

1616 Video analytics as a tool for measuring total mixed ration uniformity on dairy farms. I. G. M. A. Santos^{*1}, M. Tegeler², A. R. Reibman³, D. R. Buckmaster⁴, and J. P. Boerman¹, ¹*Department of Animal Sciences, Purdue University, West Lafayette, IN*, ²*Diamond V, Cedar Rapids, IA*, ³*School of Electrical and Computer Engineering, Purdue University, West Lafayette, IN*, ⁴*Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, IN*.

Achieving uniformity in total mixed rations (TMR) on dairy farms is crucial for optimizing rumen function, enhancing nutrient utilization, and maintaining overall animal health. The objective of this study was to explore the potential of using image analysis as a tool to assess TMR uniformity. Cameras mounted above a mixer wagon were used to record the mixing process of poorly ($n = 6$) and well-mixed ($n = 9$) lactating cow diets. Poorly mixed and well-mixed diets differed in load size and mixing time, with the poorly mixed diet having a larger load size and shorter mixing time than the well-mixed diets. The initial, middle, and final frames from each batch were obtained and converted to the red, green, and blue (RGB) color space. Within each frame, 100 regions of interest (ROI) were selected, and pairwise comparisons were made using the earth mover's distance (EMD) to quantify the uniformity of the TMR by comparing pixel intensity distribution within the ROI per frame. All diets were analyzed with the Penn State Particle Separator (PSPS) and for nutrient analysis ($n = 10$ samples per batch, $n = 15$ batches; total $n = 150$). The EMD means were calculated using Python for all possible pairwise comparisons ($n = 4,950$) between 100 ROI for each frame to assess uniformity. Batch means were analyzed using R 4.3.2 to calculate Pearson correlations between PSPS sieve distributions, mean particle size, nutrient analysis, and video-derived data. Means of individual EMD values and 5th to 95th percentile confidence intervals (CI) were calculated per frame. Mean EMD was highly positively correlated with PSPS variables and zinc and manganese concentrations ($R^2 > 0.63$; $P < 0.05$). The EMD (lower is better) moved from 28.32 to 21.40 for the poorly mixed diets and from 36.39 to 18.68 for the well-mixed diets. Correspondingly, the CI moved from 5.51–65.56 to 4.29–49.46, and from 6.11–85.58 to 4.18–40.15 for the poorly and well-mixed diets, respectively. The reduction in mean EMD variability throughout mixing for the well-mixed diets suggests that mixing improved uniformity over time, and the final differences between conditions indicate that image analysis has potential as a tool for assessing diet uniformity.

Key Words: diet uniformity, feed mixing, image processing

1617 Monitoring total mixed ration to assess mycotoxin and *Clostridium* contamination on Maryland dairy farms. N. Suresh^{*1}, J. Semler², T. Fernandes³, and F. F. Cardoso¹, ¹*Department of Animal and Avian Sciences, University of Maryland, College Park, MD*, ²*University of Maryland Extension, Boonsboro, MD*, ³*School of Animal Sciences, Virginia Tech University, Blacksburg, VA*.

Feed is the largest expense on a dairy farm, making feed efficiency crucial to the success of the operation. Optimal feed efficiency reduces costs and ensures resources are used effectively. A key component of achieving this is minimizing feed waste. Feed waste can be attributed to several factors, with contamination being a common reason. Mycotoxins and *Clostridium* have detrimental impacts on feed efficiency, animal health, and production. Due to this, there is a large concern from dairy farmers regarding mycotoxin and *Clostridium* contamination in feed. Unfortunately, farmers do not test their feed for mycotoxins and *Clostridium* as often as they would like, due to the expensive prices for testing. This study aimed to assess the prevalence of *Clostridium* and mycotoxins in total mixed rations (TMR) from 29 dairy farms across Maryland.

Samples of TMR were analyzed at Rock River Laboratory (Watertown, WI) to evaluate levels of mycotoxins and *Clostridium perfringens*. Descriptive analyses were generated using SAS to assess the distribution and variability of deoxynivalenol (DON), zearalenone, fumonisin, and *Cl. perfringens*. A frequency analysis determined the prevalence of *Cl. perfringens*, and correlation analysis evaluated potential relationships between mycotoxin levels and bacterial presence. The most prevalent mycotoxin was DON, detected in 29 farms, with concentrations ranging from 0 to 2.78 ppm (mean = 0.78 ± 0.66 ppm). Zearalenone was found in 20 farms, with levels between 0 and 116 ppb (mean = 39.88 ± 32.11 ppb). Fumonisin was detected in 21 farms, ranging from 0 to 10 ppm (mean = 1.51 ± 1.94 ppm). *Clostridium perfringens* was found in 24% of farms, with concentrations ranging from 0 to 40 cfu/g (mean = 4.48 ± 9.85 cfu/g). Pearson correlation analysis showed a significant positive correlation between DON and zearalenone ($r = 0.54$, $P < 0.01$) and between DON and *Cl. perfringens* ($r = 0.40$, $P = 0.03$). These findings show potential risks to feed quality and animal performance, providing valuable insights to guide targeted extension education and outreach. By improving feed management practices, these efforts can enhance the overall profitability of dairy operations across the state.

Key Words: mycotoxin, *Clostridium*, extension

1618 Volatile organic compound concentrations in natural and mechanically ventilated dairy farms. A. Jannat^{*}, R. Campion, B. Goncalves da Costa, and D. Manriquez, *Colorado State University, Fort Collins, CO*.

The sources and variability of volatile organic compounds (VOC) emissions in different dairy systems remain poorly understood. This study aimed to characterize indoor VOC in dairy farms with different ventilation and milking systems. Data were collected from 2 commercial dairy farms in Colorado. Farm A had 1 tunnel-ventilated barn with 2,000 lactating cows and a 90-stall rotary milking parlor. Farm B had 1 naturally ventilated barn with 400 lactating cows and an automated milking system parlor. Air samples were collected in December 2024 from Farm A and in January 2025 from Farm B, using stainless-steel canisters and a time-controlled air valve for a 24-h sampling period deployed at the barns and milking parlors of both dairies. Additionally, temperature, humidity, and wind speed were recorded using weather stations and temperature-humidity index sensors at both locations. After sampling, VOC were identified and concentrations were determined at the chemical atmospheric laboratory at Colorado State University. A total of 52 VOC were detected across both farms, ranked by concentration at each location. Most VOC were common at both farms, although some VOC were unique within farms. In both cow housing barns, methane (A: 22.32; B: 16.72 ppmv), trichloroethylene (A: 16.4; B: 5.2 ppbv), and ethane (A: 13.72; B: 6.51 ppbv) were the top 3 high-concentration VOC. In the milking parlor of Farm A, ethane (18.4 ppmv), propane (13.72 ppbv), and methane (11 ppmv) were ranked top 3; whereas methane (20.28 ppmv), ethane (6.43 ppmv), and trichloroethylene were top 3 in the milking parlor of Farm B. In addition, pentane and hexane were uniquely identified in Farm A, whereas acetonitrile and tetrachloroethylene were exclusively detected in Farm B. Because VOC can adversely impact human and animal health, causing short- and long-term effects such as airway irritation, nausea, and damage to the liver, kidneys, and central nervous system, research on VOC concentration dynamics is critical to identifying emissions sources and practices subject to mitigation, as well as to determine air quality standards for different operation systems within the US dairy industry.

Key Words: air quality, pollutant, emissions

1619 Microwave-powered dairy manure treatment method for pathogen and microbial greenhouse gas emission control. P. Pandey*, *University of California Davis, Davis, CA.*

Manure-borne pathogens and greenhouse gas (GHG) emissions from manure are often a source of concern for the dairy industry. In the dairy sector, manure is one of the major sources of GHG emissions. Manure has potential to emit methane and nitrous oxide, depending on the practices used in manure handling. Previous estimates show that about 50% of GHG emissions in the dairy sector are related to manure. In addition, manure is also linked with pathogens that have potential to cause foodborne illnesses in human. Here we have developed a novel microwave-powered method to rapidly control bacteria and moisture in manure, with potential to reduce to GHG emissions and microbial contamination caused by manure. Results showed that the microwave-based treatment technology is capable of inactivating dairy manure-borne bacteria such as *Escherichia coli* instantly, depending on the level of microwave power. Further, the heat generated by microwaves rapidly accelerates uniform moisture removal from manure, which allows the conversion of manure into value-added and microbial risk-free products. Energy generated by microwaves in manure assists in densification and compaction of manure, which can help in many ways, including transportation, waste reduction, and application of manure in cropland. We anticipate that this microwave-based advanced technology has potential for use in the dairy industry at a large scale.

Key Words: microwave, dairy manure, public health risk

1620 Evaluating composting efficacy in compost bedded pack barns across California. R. A. Black*¹, B. M. Karle², D. R. Bruno³, and R. Branco Lopes⁴, ¹*University of California Agriculture and Natural Resources, Cooperative Extension Sonoma County, Santa Rosa, CA,* ²*University of California Agriculture and Natural Resources, Cooperative Extension Glenn County, Orland, CA,* ³*University of California Agriculture and Natural Resources, Cooperative Extension Fresno County, Fresno, CA,* ⁴*University of California Agriculture and Natural Resources, Cooperative Extension Tulare County, Tulare, CA.*

Compost bedded pack (CBP) barns serve as a climate-smart manure management practice, maintaining manure in aerobic conditions compared with anaerobic manure ponds. California's Alternative Manure Management Program provides funding to dairies to construct these barns, supporting the state's ambitious greenhouse gas reduction goals. The objective of this study was to quantify composting efficacy from 9 CBP barns. Sampling occurred across 4 seasons (fall 2023, winter 2024, spring 2024, and summer 2024) and in 2 geographic regions of California: North Coast (NC, n = 3) and South Valley (SV, n = 6). The CBP barns were sampled at 3 depths (surface, 15 cm, and 30 cm) across 9 locations per CBP barn to create one composite sample for each depth, with samples analyzed for C:N ratio and moisture. Data were analyzed using a mixed linear model in SAS. Results showed that compost C:N was greatest at 30 cm (20.8 ± 0.9) compared with the surface (17.5 ± 0.8 , $P < 0.01$), but did not differ from 15 cm (19.5 ± 0.8 , $P > 0.05$). The NC region had higher C:N than the SV region (25.8 ± 1.1 vs. 12.7 ± 0.8 , respectively; $P < 0.01$). The surface of the CBP was drier ($53.0 \pm 1.4\%$) compared with 15-cm and 30-cm depths ($56.2\% \pm 1.4\%$ and $57.0\% \pm 1.4\%$, respectively; $P < 0.05$). Moisture at 15-cm and 30-cm depths did not differ. The CBP barns in the SV region were drier than in the NC region ($43.0\% \pm 1.3\%$ vs. $67.8\% \pm 1.9\%$, respectively; $P < 0.01$). Seasonal variation did not affect C:N or moisture ($P > 0.05$). The CBP barns in the SV region demonstrated greater composting efficacy, indicated by lower C:N and moisture levels, likely due to management differences. The SV barns were stirred at least monthly, whereas NC

barns were not. These findings highlight the importance of management practices, such as regular stirring, in optimizing composting efficacy in CBP barns. The lower moisture and C:N ratio observed in the SV region suggest that active management can enhance aerobic decomposition and reduce anaerobic emissions. Implementing the best practices for CBP barn management can improve manure storage efficiency and contribute to California's greenhouse gas reduction efforts.

Key Words: compost bedded pack barn, greenhouse gas reduction, manure management

1621 Mechanical manure solids separation on 19 California dairy farms. B. M. Karle*¹, R. A. Black², D. R. Bruno³, and R. Branco Lopes⁴, ¹*University of California Agriculture and Natural Resources, Cooperative Extension Glenn County, Orland, CA,* ²*University of California Agriculture and Natural Resources, Cooperative Extension Sonoma County, Santa Rosa, CA,* ³*University of California Agriculture and Natural Resources, Cooperative Extension Fresno County, Fresno, CA,* ⁴*University of California Agriculture and Natural Resources, Cooperative Extension Tulare County, Tulare, CA.*

The voluntary adoption of climate-smart agriculture practices for dairy farms through the Alternative Manure Management Program (AMMP) is a strategy toward meeting California's aggressive greenhouse gas reduction goals. With funding from AMMP and other sources, dairy producers have installed mechanical manure separation systems as one method to reduce methane emissions. The objective of this study was to quantify the removal of total and volatile solids across seasons. Liquid influent and both liquid and solid effluent from 19 mechanical manure separators, including sloped screen (n = 11), conveyor (n = 5), and screw press (n = 3), were sampled. Sampling occurred across 4 seasons (fall 2023, winter 2024, spring 2024, and summer 2024) and in 3 geographic regions of California: North Coast (NC, n = 6), North Valley (NV, n = 5), and South Valley (SV, n = 8). Liquid samples were analyzed for total solids (TS) and volatile solids (VS), and solid samples for dry matter content. Each dairy farm completed a questionnaire on manure management and separator use. Data were analyzed using a mixed linear model in SAS. Reduction of VS and TS was less in the NV region ($13.3\% \pm 3.7\%$ and $11.2\% \pm 3.2\%$, respectively) compared with the NC ($33.5\% \pm 3.0\%$ and $25.6\% \pm 2.6\%$, respectively) and SV regions ($27.6\% \pm 3.2\%$ and $24.5\% \pm 2.8\%$ respectively; $P < 0.01$). Season, manure collection system, and separator type did not significantly affect VS or TS removal ($P > 0.05$). Conveyor manure separation systems produced manure solids higher in moisture ($82.9\% \pm 1.3\%$) compared with sloped screen ($78.3\% \pm 1.5\%$) and screw press manure separation systems ($73.3\% \pm 2.0\%$; $P < 0.01$). The SV region used separation systems that removed more moisture from solids than the NC and NV regions ($72.2\% \pm 1.3\%$ vs. $80.9\% \pm 1.1\%$ and $81.4\% \pm 1.4\%$, respectively; $P < 0.01$). Separated manure solids moisture was not affected by manure collection system or season ($P > 0.05$). Regional manure management on dairies likely plays a key role in differences in manure separation efficiency. Selecting a system that performs best in the region of implementation may increase performance metrics and producer satisfaction with the system.

Key Words: manure, separator, California

1622 Environment as challenge for intensive dairy farming. A. Kuipers*, P. Galama, and P. W. G. Groot Koerkamp, *Wageningen University & Research, Wageningen, the Netherlands.*

Objectives were to perform a feasibility study to evaluate techniques for both NH_3 and CH_4 reduction from dairy barns, especially air filtering

and treatment techniques, as requested by the Dutch Ministry of Agriculture, Fisheries, Food Security and Nature. Techniques for reducing NH_3 emissions are being evaluated in case control studies of Wageningen University & Research (UR), as well as a few other designated institutes, in combination with commercial farms. A list of floor types and manure management techniques was recently made available with the established emission factors. A base emission of 13 kg NH_3 /cow place per year is used. The various floor systems and manure handling techniques are researched at Wageningen UR in small, controlled barn units, with cows and the measured NH_3 emissions compared with this 13 kg NH_3 /cow place per year. As examples, the Cow Toilet shows a 35% reduction, and the Lely Sphere a 75% reduction in NH_3 output compared with the standard. In addition, we studied combined mitigation of CH_4 and NH_3 from dairy barns through barn design, ventilation, and air treatment systems. Focus was on enteric CH_4 , because it represents around 80% of CH_4 output at the dairy farm. In 60 barns across Europe, we measured average concentrations of 5 to 80 ppm over a 1-yr period with a simplified method. This method used a FlexFoil sample bag to collect air during a structured walk through the barn of 30 min in between feeding times. The collected air goes to the laboratory for measurement

of gases such as CH_4 . Present capturing and oxidizing techniques only work with higher concentrations of CH_4 . Therefore, experiments collecting air at specific locations (close to cows' mouths) or mixed with air from a closed manure storage (with 60% CH_4 in headspace) to increase concentrations were performed. Using a hood construction above cows resulted in CH_4 concentrations of mean 125 and SD 56 ppm. With lower temperatures and more closed buildings, higher concentrations were achieved, up to 700 ppm. Acidification of manure reduced emissions of both gases (30%–40% reduction in NH_3 ; 40%–90% reduction in CH_4). Valorization of the filtered N and C components is crucial for economic reason. Renure (REcovered Nitrogen from manURE) offers opportunities to value manure, as does production of green gas (biomethane). To conclude our study, we developed 3 barn designs to picture potential housing systems that combine all useful practices and techniques to reduce emissions and validate the obtained manure products. In general, a policy shift is envisaged from means regulations to target regulations, providing farmers with their own capacity to reach the prescribed targets.

Key Words: emissions, dairy barn, air filtering

Ruminant Nutrition 7: Methane

1623 Identification of anti-methanogenic compounds using in silico docking and in vitro ruminal fermentation techniques. S. Zhu^{*1,2}, G. Foggi¹, R. Peng¹, S. Riniker³, and M. Niu¹, ¹*Animal Nutrition, Institute of Agricultural Sciences, Department of Environmental Systems Science, ETH Zürich, Zurich, Switzerland*, ²*Zhejiang University, Hangzhou, Zhejiang, China*, ³*Institute of Molecular Physical Science, Department of Chemistry and Applied Biosciences, ETH Zurich, Zurich, Switzerland*.

The pressure on livestock systems to reduce their environmental impact is growing, with dairy cows being a major methane source. Several studies have screened compounds for methanogenesis inhibition, yet research on targeting the enzymatic activity of methanogenic archaea, particularly methyl-coenzyme M reductase (MCR), remains limited. This study combined molecular docking to identify potential MCR inhibitors from natural compounds with in vitro validation of selected inhibitors' anti-methanogenic potential. Our in silico screening involved more than 180,000 compounds from plant, food, yeast, milk, and bovine-related databases. Compounds with undesirable properties, including pan assay interference compounds (PAINS) and poor absorption, distribution, metabolism, and excretion (ADMET) profiles, were filtered before docking. Potential MCR binders were predicted using AutoDock Vina, followed by molecular docking with the high-resolution MCR crystal structure (PDB ID: 5A0Y, 1.10 Å), specifically targeting the methyl-CoM and CoB binding pocket (center x = 28.172, y = 37.21, z = -53; size 20 × 20 × 20 Å). To improve selectivity and reduce off-target effects, compounds interacting with 7 enzymes from glycolysis and short-chain fatty acid pathways were excluded. A total of 896 potential inhibitors, including bisdemethoxycurcumin, paeoniflorin, and mulberroside F, were identified using a binding affinity cutoff of -8.0 kcal/mol, with the lowest value equal to -12.3 kcal/mol (rutaretin 9-rutinoside). The criteria for selecting the compounds were as follows: binding energy (affinity), availability on the market, cost-effectiveness of the product when available, and, finally, number of hydrogen bonds. Bisdemethoxycurcumin, a polyphenol from turmeric, showed a binding affinity of -9.6 kcal/mol with 2 hydrogen bonds and 7 active torsions. Paeoniflorin, a terpene glycoside from peony, had -9.1 kcal/mol affinity with 13 torsions. Mulberroside F, a flavonoid from mulberry leaves, exhibited -9.9 kcal/mol affinity with 2 hydrogen bonds and 16 torsions. All compounds, sorted according to binding energy, were screened to achieve a minimum of 24 compounds for anti-methanogenic in vitro testing, based on the listed criteria. Further work will include assembling a database of candidate compounds with anti-methanogenic properties.

Key Words: methanogenesis inhibition, virtual screening, molecular docking, methyl-coenzyme M reductase (MCR), active compounds

1624 Using in vitro methane emitting value of feeds to predict in vivo methane production in dairy cattle. C. V. Almeida^{*1}, C. J. Eifert¹, L. F. Martins¹, S. Issabekova¹, N. Stepanchenko¹, S. F. Cueva^{1,2}, Y. Kim¹, A. Richards¹, D. E. Richards¹, and A. N. Hristov¹, ¹*The Pennsylvania State University, University Park, PA*, ²*Cargill Animal Nutrition, Shippensburg, PA*.

Compared with in vivo experiments, in vitro fermentation systems may offer a faster and less expensive option to evaluate the effects of feeds and complete diets on enteric CH₄ emission in ruminants. The objectives of this study were (1) to analyze the in vitro CH₄ emitting value (MEV) of feeds often used in dairy cattle diets, and (2) to evaluate the possibil-

ity of predicting in vivo CH₄ emission from complete diets based on MEV of individual feeds. Two ruminally cannulated lactating Holstein cows fed a standard 52% forages and 48% concentrate feeds diet were used as donors of rumen inoculum. Thirty-five incubations were carried out for 48 h at 39°C with continuous agitation using an automated gas production system. Incubations included forages and concentrate feeds (n = 26) and TMR (n = 22), dried and ground through a 0.5-mm sieve, collected from 10 experiments with lactating dairy cows where enteric CH₄ production was measured using the GreenFeed system. Total gas production was continuously monitored, and headspace samples were analyzed at 12, 24, and 48 h for CH₄ concentration. Data were analyzed using the MIXED procedure of SAS with the fixed effect of feed and the random effect of incubation included in the model. Overall, feeds containing high concentrations of digestible carbohydrates had greater ($P < 0.01$) MEV, expressed as mL/g feed DM. Linseed meal and beet pulp had the greatest ($P < 0.01$) and whole cottonseed had the lowest ($P < 0.01$) MEV, expressed as percentage of total gas. Cottonseed hulls and beet pulp had the highest ($P < 0.01$) molar proportion of acetate, whereas oat grain had the highest ($P < 0.01$) molar proportion of propionate. The MEV of reconstituted diets was weakly related to in vivo enteric CH₄ production in lactating dairy cows ($R^2 = 0.20$, $P = 0.01$), but there was a strong relationship between MEV of TMR evaluated in vitro and in vivo ($R^2 = 0.79$, $P = 0.001$). In this study, MEV of individual feeds did not predict well in vivo enteric CH₄ emissions of diets based on these feeds, but there was a good relationship between in vivo enteric CH₄ emission and in vitro MEV of complete diets.

Key Words: dairy feed, methane production, in vitro

1625 Effects of long-term *Asparagopsis taxiformis* supplementation on enteric methane emission and lactational performance of dairy cows. S. Issabekova^{*1}, L. F. Martins¹, N. Stepanchenko¹, C. V. Almeida¹, C. J. Eifert¹, A. Richards¹, D. E. Wasson¹, S. F. Cueva², and A. N. Hristov¹, ¹*The Pennsylvania State University, University Park, PA*, ²*Cargill Animal Nutrition, Shippensburg, PA*.

Asparagopsis taxiformis (AT) has been shown to decrease enteric CH₄ emissions in ruminants, but long-term data with AT-based additives are lacking. The objective of this study was to evaluate the long-term effects of AT on enteric CH₄ emission and performance of lactating dairy cows. Forty Holstein cows (averaging 3.0 lactations, 33 DIM, and 47 kg/d milk yield [MY]), housed in 2 freestall pens (20 cows per treatment), were enrolled in a 39-wk randomized complete block design experiment with a 2-wk covariate period and 37 wk for data collection. Treatments were control (CON, basal diet) and CON supplemented with 0.25% AT (DM basis; AT). Daily enteric CH₄ emission was measured continuously using 2 GreenFeed units. Pen DMI, MY, and BW of the cows were recorded daily, and samples for milk component analyses were collected monthly. Data were analyzed using the MIXED procedure of SAS, with fixed effects of treatment, week, and treatment × week in the model. Compared with CON, the overall (study wk 7 to 39) mitigation effect of AT on daily CH₄ and CH₄ emission intensity was 28% ($P = 0.009$; 353 vs. 273 g/d and 10.8 vs. 7.4 g/kg MY for CON and AT, respectively), although it was inconsistent throughout the duration of the study. Relative to CON, AT decreased ($P \leq 0.001$) pen DMI (27.0 vs. 23.2 kg/d), but MY and ECM yield were not affected by treatment (38.2 vs. 37.6 and 38.4 vs. 39.0 kg/d, respectively), which resulted in increased ($P \leq 0.01$) feed efficiency on both MY and ECM basis, compared with CON. Milk composition was also not affected by AT, but BW, BW gain, and BCS of the cows

were lower ($P \leq 0.03$) for AT (692 kg, 0.28 kg/d, and 3.19) vs. CON (722 kg, 0.49 kg/d, and 3.30), respectively. Concentrations of bromide and iodine in milk from AT cows were 26 and 5.7 times greater ($P \leq 0.002$) at the end of the study compared with the covariate period. In the long term, feeding AT at 0.25% of dietary DM substantially decreased enteric CH₄ emission metrics in dairy cows without a significant effect on milk production or composition and increased feed efficiency, but cows experienced losses in BW and BCS.

Key Words: methane, *Asparagopsis taxiformis*, dairy cow

1626 Evaluating a tank-grown *Asparagopsis taxiformis* variety (Brominata) for health and safety in dairy cows. S. E. Omale* and J. A. D. R. N. Appuhamy, *Department of Animal Science, Iowa State University, Ames, IA.*

Despite the unprecedented methane mitigation efficacy of *Asparagopsis taxiformis* (AT), lack of toxicity and milk quality studies has precluded the widespread approval of AT in dairy cow diets. Brominata is an AT variety registered in Mexico and has been fed at an inclusion rate (IR) of 0.30% of DM (SIR). The study objective was to evaluate the safety and tolerance of Brominata fed at SIR or higher IR. Forty-eight late-lactation Holstein cows were fed freeze-dried Brominata at 0% (CTL), SIR, 0.45%, or 0.60% of DM for 9 wk after a 4-wk IR ramp-up phase. Brominata residues and somatic cell counts (SCC) in milk were determined fortnightly. Body condition score (BCS), locomotion, breathing, fecal consistency (FC), and neurological disorders (NDS) were monitored weekly. Blood from wk 5 and 9 was analyzed for chemistry and hematology. Rumination time (RT, min/kg of DMI) was recorded daily. Four cows per treatment were slaughtered, and tissues were harvested for pathological analyses. Data were analyzed using the MIXED procedure of SAS. Brominata IR linearly decreased DMI but increased RT ($P < 0.01$). Cows' SCC, BCS, locomotion, respiration, FC, NDS, and hematology ($P > 0.05$) were not altered. Blood alanine transaminase, cholesterol, and albumin decreased in response to Brominata IR ($P < 0.05$). Milk bromoform, bromide, and iodine levels increased linearly with IR ($P < 0.01$). Milk bromoform (~0.002 ppm) was lower than the threshold (0.080 ppm). Milk iodine (~1.10 ppm) was above the upper limit (0.83 ppm) of the reference range due to low milk yields of late lactation and high baseline iodine (0.37 ppm). Gross pathology examinations revealed elevated papillary blunting and mucosal scarring in the rumen ($P = 0.02$), although histological examinations showed no differences in papillary length and mucosal thickness. No abnormality was observed in the small intestine, colon, thyroid, thymus, kidney, liver, or brain. The results indicated that Brominata did not adversely affect cow health and milk safety; further analyses, representing various stages of lactation, may help to draw more robust conclusions.

Key Words: bromoform, iodine, tissue pathology

1627 Effect of a novel inhibitor on enteric methane emissions and lactational performance of Holstein cows. A. Richards*, C. Eifert, L. F. Martins, N. Stepanchenko, C. Vidal de Almeida, D. Wasson, S. Issabekova, O. Pitkanen, E. Ramirez Bribiesca, Y. Kim, and A. N. Hristov, *The Pennsylvania State University, State College, PA.*

This study was undertaken to test the effect of a novel methane inhibitor, AB-01, on enteric CH₄ emission and performance of mid-lactation Holstein cows. A total of 48 cows, averaging 87 d in milk (DIM) and milk yield (MY) of 43 kg/d were used in the study. The experiment was a randomized complete block design, with 2-wk covariate and 12-wk

data collection periods. Cows were blocked by parity, DIM, and MY during the covariate period and randomly assigned to 1 of 4 treatments: (1) CON, basal diet; (2) LOW, CON supplemented with AB-01 at 15 mg/kg feed DM; (3) MED, CON supplemented with AB-01 at 25 mg/kg; and (4) HIGH, CON supplemented with AB-01 at 35 mg/kg of DM. Enteric gas emission data were continuously measured using 2 GreenFeed units. Data were analyzed using the Mixed procedure of SAS. Polynomial contrasts were used to determine linear and quadratic effects of treatment. As AB-01 dose increased, enteric CH₄ emission (371, 298, 241, and 231 g/d for CON, LOW, MED, and HIGH, respectively), yield (CH₄/DMI; 13.9, 11.7, 10.1, and 11.2 g/kg, respectively), and intensity (CH₄/energy-corrected milk [ECM]; 9.90, 7.62, 6.35, and 6.42 g/kg, respectively) decreased linearly ($P < 0.001$). Increasing AB-01 dose linearly increased ($P < 0.001$) H₂ emission (2.06, 5.95, 7.48, and 8.63 g/d, respectively). Increasing AB-01 inclusion rate linearly decreased ($P < 0.001$) DMI (26.8, 26.3, 24.4, and 21.5 kg/d, respectively). Milk yield, ECM yield (38.9, 38.1, 36.9, and 35.6 kg/d, respectively), and yields of milk true protein, lactose, and total solids also decreased linearly ($P \leq 0.05$) with increasing AB-01 inclusion rate. Both ECM feed efficiency (1.47, 1.53, 1.60, and 1.68 kg/kg, respectively) and milk urea nitrogen increased linearly ($P \leq 0.01$) with increasing AB-01 dose. Increasing AB-01 dose also linearly decreased ($P \leq 0.03$) body weight and body condition score of the cows. In conclusion, AB-01 supplementation decreased enteric CH₄ emission yield by up to 38% and improved feed efficiency in lactating Holstein cows; however, feed intake, milk production, and body condition of the cows were negatively affected.

Key Words: milk production, enteric methane, inhibitor

1628 Effects of whey and 3-nitroxypropanol (3-NOP) supplementation on lactational performance and enteric methane in dairy cows. K. Wang*, U. Arshad, X. Ma, Z. Islam, R. Peng, S. Zhu, M. B. Blanco, S. Bagaria, C. N. Major, M. Wang, E. Colella, C. Ruegg, G. Foggi, Y. Li, M. Niu, *Animal Nutrition, Institute of Agricultural Sciences, Department of Environmental Systems Science, ETH Zürich, Zurich, Switzerland.*

Whey, a lactose-rich byproduct, may foster lactic acid bacteria linked to low CH₄ phenotypes because lactose fermentation yields lactate, convertible to propionate, reducing H₂ available for CH₄ synthesis. An in vitro study suggested that whey and 3-nitroxypropanol (3-NOP) synergistically reduced CH₄ production by 48%, vs. 28% with 3-NOP alone. This study evaluated whey and 3-NOP supplementation in 24 Holstein cows (177 ± 46 DIM) assigned in a 2 × 2 factorial design to either a basal (BAS) or a whey-mixed (WHM; 15% DM) diet supplemented with placebo (PCB) or 3-NOP (80 mg/kg TMR DM). The 13-wk study included a 1-wk covariate period on the BAS diet followed by 12 wk on the experimental diets. Diets were balanced for CP (15.4%), starch (18.3%), and NDF (38.3% of TMR DM). Cows were fed and milked 2×/d, and gas emissions were measured using GreenFeed. Data were analyzed with mixed models, including fixed effects of diet (BAS vs. WHM), inhibitor (PCB vs. 3-NOP), week, and their interactions, and a random effect of cow. A tendency ($P = 0.10$) for a diet × inhibitor interaction was observed for DMI, because WHM cows receiving PCB had 2.3 kg DM/d lower DMI than BAS cows. Milk yield and composition were unaffected. No diet × inhibitor interaction was detected for CH₄ emissions. Feeding WHM ($P = 0.05$) reduced CH₄ production by 8.3%, whereas 3-NOP ($P < 0.01$) reduced production by 38.7% and decreased CH₄ yield and intensity by 34.5% and 40.7%, respectively; nevertheless, WHM had no effect on CH₄ yield or intensity. A tendency for diet × inhibitor interaction ($P = 0.09$) was found for H₂ production: WHM ($P < 0.01$) and 3-NOP ($P < 0.01$) increased H₂ production by 81.2% and

150%, respectively, with their combination yielding a 298% increase. Both WHM ($P < 0.01$) and 3-NOP ($P < 0.01$) increased H_2 yield by 70.5% and 120%, respectively. In conclusion, no in vivo synergy was observed between 3-NOP and WHM for reducing CH_4 emissions. Whey reduced CH_4 production, likely due to lower feed intake, but did not improve lactational performance or reduce CH_4 yield or intensity. The effect of whey and its interaction with 3-NOP on H_2 warrants further investigation.

Key Words: whey, 3-nitroxypopropanol (3-NOP), methane mitigation

1629 Effects of oregano oil on ruminal in vitro total gas and methane production. A. Beckmann^{*1}, T. Borchardt¹, and C. Cruywagen², ¹Dostofarm GmbH, Westerstede, Germany, ²Department of Animal Sciences, Stellenbosch University, Stellenbosch, South Africa.

Methane production of dairy cows is not only disadvantageous for the animal due to loss of energy, but it is also an environmental issue. The objective of this study was to investigate the effect of oregano essential oil (OEO; DOSTO Liquid, Dostofarm GmbH, Westerstede, Germany) on ruminal in vitro total gas (TG) and methane (MET) production. Rumen fluid samples were collected from 2 nonlactating rumen-cannulated Dexter cows in the morning, immediately before the start of each run. Substrate samples (1 g ± 5 mg) consisting of 60% ground lucerne hay, 10% ground oat hay, 25% maize meal, and 5% soybean oil cake meal, were weighed out into 200-mL glass digesters. Volumes of 40 mL of rumen fluid inoculant and 160 mL of buffered incubation medium were added to each digester. The Gas Endeavor System (BPC Instruments) was used, and during the 48-h incubation period, gas measurements were recorded every 5 min. Dosages of 0, 50, 100, and 200 µL of OEO per 100 mL of incubation medium were examined in 6 in vitro runs. Results were analyzed with ANOVA, using Statistica 14. Multiple comparisons (Fisher's least significant difference) were made post hoc to discern differences among treatments, with a significance of $P < 0.05$. Measured against the negative control, TG production was increased in the 100- and 200-µL treatments, and MET production was decreased at all the OEO dilution levels (Table 1). When both TG and MET production are considered, it was concluded that rumen fermentation appeared to be improved at a dosage of 100 µL of OEO per 100 mL of incubation medium. Further research is needed to identify the mechanisms by which OEO inhibits methane production in the rumen, such as studies involving dose-response trials on shifts in the rumen microbiome, and volatile fatty acid production and ratios.

Key Words: methane, oregano essential oil, total gas

1631 Seaweeds for cows fed canola meal or soybean meal: Effects on enteric methane emissions. C. Benchaar^{*1}, F. Hassanat², and S. Terry³, ¹Agriculture and Agri-Food Canada, Sherbrooke Research and Development Centre, Sherbrooke, QC, Canada, ²Agriculture and Agri-Food Canada, Quebec Research and Development Centre, Quebec, QC, Canada, ³Agriculture and Agri-Food Canada,

Lethbridge Research and Development Centre, Lethbridge, AB, Canada.

In 2 previous studies, we showed that replacing soybean meal (SBM) with canola meal (CM) and feeding seaweed reduced enteric CH_4 production (g/d), yield (g/kg of DMI), or emission intensity (g/kg ECM). The objective of this study was to assess the potential of combining these 2 dietary strategies on enteric CH_4 production and performance in lactating dairy cows. Twelve lactating, multiparous Holstein cows (DIM = 115 ± 16; milk yield = 46.1 ± 5.4 kg) were used in a replicated 4 × 4 Latin square (35-d period; 14-d adaptation) with a 2 × 2 factorial arrangement of treatments. Cows were fed (ad libitum, 5% orts) a TMR (52:48 forage:concentrate ratio on a DM basis) containing CM or SBM (as the main protein source) without or with the addition (0.18%, DM basis) of seaweed (Synergraze Inc., Calgary, AB, Canada). Canola meal (25% of diet DM) was included in the TMR at the expense of SBM and soybean hulls. Milk production and composition (7 d) and CH_4 production data (respiratory chambers, 3 d) were analyze using the MIXED procedure of SAS to determine the effects of meal, seaweed, and their interactions. Significance was declared at $P \leq 0.05$. Feeding cows the seaweed mixture reduced DMI by 6%. Also, feeding seaweed reduced (6%) milk production (ECM kg/d) in cows fed CM but not in cows fed SBM (meal × seaweed interaction, $P = 0.02$). Feeding seaweed decreased CH_4 production (g/d), yield (g/kg DMI), and intensity (g/kg ECM) by 33%, on average. Compared with feeding SBM without seaweed mixture, feeding CM and seaweed reduced enteric CH_4 production, yield, and intensity by 41%, 39%, and 38%, respectively. This study shows that combining CM and a seaweed in dairy cow diets is an effective strategy to achieve substantial reductions in enteric CH_4 production and emission intensity.

Key Words: seaweed, canola meal, methane

1632 Grass silage versus corn silage in nutritionally balanced diets: Effects on methane emissions in mid-lactating dairy cows. M. van Tol^{*1,2}, W. M. van Straalen², B. Kemp¹, J. B. Roelofs¹, and A. T. M. van Kneegsel¹, ¹Adaptation Physiology Group, Wageningen University, Wageningen, the Netherlands, ²Schothorst Feed Research, Lelystad, the Netherlands.

In previous studies, the effect of diets consisting mainly of grass silage or corn silage on methane (CH_4) emission was measured. However, these diets included high proportions of roughage (up to 80% of dry matter [DM]), which do not fully meet the nutritional requirements for milk production. The aim of this study was to evaluate the effects of feeding a diet formulated to meet all nutritional requirements (according to CVB Feed Table), with grass silage or corn silage as the main roughage component, on CH_4 emission. Thirty-two Holstein-Friesian mid-lactation cows were blocked based on parity and fat- and protein-corrected milk (FPCM) yield and offered a diet with mainly forage grass silage (GS, n = 16) or corn silage (CS, n = 16). Diets consisted of 40% concentrate feed and either 48% grass silage and 12% corn silage (GS) or 48% corn

Table 1 (Abstr. 1629). Effects of different OEO dosages on ruminal in vitro total gas and methane production after 48 h of incubation

Parameter	OEO (µL/100 mL incubation medium)				P-value
	0	50	100	200	
TG (mL)	185 ^a	195 ^{ad}	212 ^c	200 ^{bd}	<0.01
MET (mL)	108 ^a	97 ^b	88 ^c	91 ^{bc}	<0.01
MET (% of TG)	58.5 ^a	49.5 ^b	41.5 ^c	45.5 ^{bc}	<0.001

Table 1 (Abstr. 1632). Chemical composition of the experimental diets

Chemical composition (g/kg DM)	Grass silage diet	Corn silage diet
DM (g/kg of product)	459	462
Crude protein	167	168
Crude fat	44	41
Sugar	67	62
Starch	135	198
NDF	389	337

silage and 12% grass silage (CS). Two concentrate feeds were formulated for GS and CS groups, to meet nutritional requirements for milk production. The chemical compositions of the diets are presented in Table 1. Cow-individual DM intake (DMI) and milk production were measured, and CH₄ and carbon dioxide (CO₂) emission quantified in a GreenFeed system. Data were analyzed using the REML procedure in GenStat. The CH₄ emission, DMI, and FPCM yield did not differ between treatments. The CO₂ yield was lower for the CS than for the GS group (517 vs. 559 g/kg DMI, $P \leq 0.04$), whereas CO₂ production (g/d) or CO₂ intensity (g/kg FPCM) did not differ. In conclusion, CH₄ emission did not differ between GS and CS diet when formulated to meet the requirements for milk production and when providing 40% of concentrate.

Key Words: grass silage, corn silage, methane emission

2519 Essential oils, when supplied in the feed or via a slow-release rumen bolus, reduce methane output and alter the milk fatty acid profile of dairy cows. C. E. M. Roxborough^{*1}, A. M. Mackenzie¹, J. Bennison², and L. A. Sinclair¹, ¹*Harper Adams University, Newport, Shropshire, UK*, ²*Agrimin Ltd., Kirmington, Lincolnshire, UK*.

Essential oils are volatile compounds consisting of plant secondary metabolites that have been reported to manipulate rumen fermentation, which can reduce methane (CH₄) emissions and alter the milk fatty acid (MFA) profile. Providing essential oils in a slow-release rumen bolus is an easier means to administer, particularly for extensively grazed ruminants. The objective of the study was to examine the effect of

essential oils when supplied in a slow-release rumen bolus or in the diet on CH₄ emissions and MFA profile of mid-lactation dairy cows. Forty-five dairy cows that were (mean $\pm$ SE) 142 $\pm$ 3.9 d in milk (DIM) and yielding 41.8 $\pm$ 1.09 kg milk/d were blocked based on DIM and milk yield and randomly assigned within block to 1 of 3 dietary treatments: C = basal total mixed ration (TMR); TO = basal TMR + essential oils (Agolin, Switzerland fed at 0.92 g/cow per day); or BO = basal TMR + intra-ruminal boluses containing essential oils to provide the same daily rate of release as TO. Individual intake was recorded daily, and milk samples collected weekly for milk composition analysis for 10 wk. Enteric CH₄ emissions were measured daily using 4 GreenFeed units. The data were analyzed as a mixed effect model, with time, treatment, lactation number, DIM, and wk 0 data as fixed effects. The daily CH₄ g/d was lower ($P < 0.001$) in cows receiving BO or TO compared with C, with means of 473, 470, and 492 g/d, respectively, an effect that was greater after wk 5 of the study. Emissions of CH₄ were also lower ($P < 0.001$) in cows on BO or TO compared with C, when expressed as g CH₄/kg milk and g CH₄/kg DMI. Concentrations of C14:1 and C16:1 were higher ($P = 0.048$ and $P = 0.004$, respectively) in cows receiving TO compared with BO, with C being intermediate. In contrast, milk C6:0 was higher ($P = 0.037$) in cows receiving BO than TO. In conclusion, supplying essential oils in the TMR or via a slow-release rumen bolus reduces CH₄ emissions from dairy cows and results in minor modifications to the MFA profile. The authors thank Agrimin Ltd. for funding the study and providing the rumen boluses.

Key Words: essential oil, bolus, methane inhibitor

ADSA-GSD Competition: Production (MS) Poster Semifinalists

2000 Effect of milk replacer and starter composition on health and immune responses of Holstein-Angus calves. M. Kovacs^{*1}, T. Chapelain¹, S. Cartwright^{1,2}, D. J. Seymour^{1,3}, D. L. Renaud⁴, and M. A. Steele¹, ¹*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada*, ²*Holstein Canada, Brantford, ON, Canada*, ³*Trouw Nutrition R&D, Boxmeer, the Netherlands*, ⁴*Department of Population Medicine, University of Guelph, Guelph, ON, Canada*.

The objective of this study was to explore the effects of pre- and post-weaning diets on the health and immune responses of calves. Male Holstein-Angus calves ($n = 68$) were enrolled at 1 wk of age and randomly assigned to 1 of 4 treatments in a 2×2 factorial arrangement: (1) lactose milk replacer (MR; 18% fat; 51% lactose) and control starter (3% crude fat; LC); (2) lactose MR and fat supplemented starter (7% crude fat; LF); (3) fat MR (29% fat; 38% lactose) and control starter (FC); and (4) fat MR and fat supplemented starter (FF). Milk replacer was mixed to balance energy and protein supply between treatments and fed at 15% of arrival BW until wk 3, and 18% thereafter, until gradual step-down weaning, which occurred from wk 6 to 10. On wk 5, calves had ad libitum access to starter feed for the remainder of the experiment. Respiratory health scores were evaluated daily for the first 13 wk using the UC Davis Respiratory Scoring chart. On wk 5 (before starter introduction), 13, and 21, calves received an intradermal injection of phytohemagglutinin (PHA) in the right tail fold and phosphate-buffered saline (PBS) in the left. Changes in skin fold thickness (SFT) were measured in triplicate using calipers at 0, 2, 4, 8, 12, and 24 h to assess cell-mediated immune responses. Data were analyzed using the GLIMMIX procedures in SAS v 9.4, considering the fixed effects of milk, starter, and their interactions, and the random effects of block and calf nested within block. Repeated measures were considered for the SFT. On wk 5, calves fed lactose MR tended to have greater changes in SFT and therefore greater localized immune responses to PHA than calves fed fat MR ($P = 0.07$). On wk 21, LC calves had the greatest change in SFT, whereas FC calves had the smallest ($P = 0.01$). No differences in SFT were found on wk 13. Between wk 5 and 13, after starter feed was introduced, the FF calves tended to have a greater proportion of days with BRD than FC calves (OR: 2.33, $P = 0.07$). Although this supports the idea that diet composition influences the measured immune response, further research is needed to explore the implications for calf health.

Key Words: calf, immune response, health

2001 Condition of calves on arrival at dairy-beef farms in the Midwestern United States. E. M. Schafer^{*1}, K. C. Creutzinger², M. M. Woodrum Setser², M. Pinkerton¹, G. G. Habing³, M. A. Erasmus¹, K. D. Vogel⁴, and J. A. Pempek⁵, ¹*Purdue University, West Lafayette, IN*, ²*University of Vermont, Burlington, VT*, ³*The Ohio State University, Columbus, OH*, ⁴*University of Wisconsin–River Falls, River Falls, WI*, ⁵*USDA-ARS Livestock Behavior Research Unit, West Lafayette, IN*.

Non-replacement dairy calves are often sold in the first days of life with limited preconditioning, and experience multiple transport events, increasing their risk of disease as they enter dairy-beef raising systems. The objective of this study was to evaluate the condition of dairy-beef calves on arrival at facilities in the Midwestern United States. From February 2024 to January 2025, 72 calves per farm (~3–7 d of age) were enrolled 6.4 ± 7.2 h after arrival at 20 dairy-beef facilities ($n = 1,436$ calves) in Indiana, Ohio, Wisconsin, and Iowa. Calves were assessed for signs of depression, dehydration, diarrhea, navel inflam-

mation, and respiratory disease. Blood samples were collected from all calves to measure serum total protein (STP) as an indicator of failed transfer of passive immunity (FTPI). On a subset of farms ($n = 11$) where blood sampling immediately after calf arrival at the farm was possible, metabolites of 30 calves per farm ($n = 327$) were analyzed for hematocrit, electrolytes, glucose, blood urea nitrogen (BUN), creatinine, and nonesterified fatty acids (NEFA). Descriptive statistics were used to estimate the prevalence of abnormal measures. Given clustering within farms, 95% confidence intervals (CI) for prevalence estimates were calculated using PROC SURVEYFREQ in SAS 9.4, with farm specified as the cluster variable. Pearson correlations were identified using PROC CORR. On arrival, 48.3% (95% CI: 38.6%–58.1%) of calves had diarrhea, 35.8% (28.6%–42.9%) had dehydration, 22.0% (16.5%–27.5%) had navel inflammation, and 18.7% (14.9%–22.6%) had depression. Blood analyses indicated 21.6% (16.3%–26.9%; cut-point STP < 5.1 g/dL) of calves had FTPI, 48.3% (29.0%–67.7%; glucose < 4.4 mmol/L) were hypoglycemic, and 31.9% (18.9%–44.8%; > 0.72 mmol/L) had elevated NEFA. There was a moderate negative correlation between NEFA and glucose ($r = -0.4$; $P < 0.001$) and a moderate positive correlation between creatinine and BUN ($r = 0.4$; $P < 0.001$). These results indicate that a high percentage of dairy-beef calves in the Midwestern United States arrive at facilities with FTPI, low energy reserves, and signs of disease that may impact long-term welfare and productivity.

Key Words: calf health, dairy-beef, blood metabolites

2002 Meta-analysis of reported methane production estimated heritabilities from predicted and direct measures: Preliminary results. T. H. Houghton^{*1}, E. M. Leishman^{1,2}, N. T. Richardson¹, J. L. Ellis³, F. Miglior^{1,4}, F. S. Schenkel¹, C. F. Baes¹, and R. E. Jahnel¹, ¹*Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada*, ²*Department of Animal and Veterinary Sciences, Aarhus University, Aarhus, Tjele, Denmark*, ³*Centre for Nutrition Modelling, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada*, ⁴*Lactanet, Guelph, ON, Canada*.

Microbial fermentation in the bovine rumen is responsible for the production of various enteric greenhouse gases. In the literature, genetic selection has been identified as a way to reduce methane production in cattle. The objective of this study was to conduct a meta-analysis to compare genetic heritability parameter estimates of methane production from different cattle types (beef or dairy), alternate collection methods (SF6, GreenFeed, respiration chambers, or sniffers), and geographic location (North America, Australia, or Europe). This was done by consolidating heritability estimates across published literature to identify factors influencing these estimates. A systematic literature review was conducted and identified 29 heritability estimates from predicted and directly measured methane production in both beef and dairy across 24 studies that met the inclusion criterion (included published standard errors [SE]). An average moderate heritability of 0.24 ± 0.01 was found across all studies. A generalized linear fixed effects trivariate model assuming a beta distribution of the estimates and a logit link function was fit using SAS 9.4 Proc. GLIMMIX, using SE of the heritability estimates as a weighting variable to determine the effects of collection method, cattle type, and geographic location on the heritability of methane production. Preliminary results show the fixed effects of collection method ($P = 0.0043$), cattle type ($P < 0.0001$), and geographic location ($P < 0.0001$) were all significant. Beef was found to have higher heritability

estimates compared with dairy, whereas estimates from North America were higher than Australia and Europe. Due to the structure of the data set, which was not well cross-classified across the levels of the factors, possible 2-way interactions were not tested, although further analysis is required. Nevertheless, the findings suggest that there can be significant impacts of collection method, cattle type, and geographic locations on the estimated heritability of methane production.

Key Words: enteric methane, cattle, genetic parameter

2003 Association of breed with health and growth performance of preweaning Holstein and beef $\times$ dairy calves. C. Rocha*, D. M. Moreira, R. E. Aguirre, S. Gill, M. M. Baugher, M. A. Ballou, and V. S. Machado, *Department of Veterinary Science, Texas Tech University, Lubbock, TX.*

As beef-on-dairy (B $\times$ D) calves become more widespread in calf-raising facilities, understanding health and growth performance differences between Holstein and B $\times$ D calves is essential for optimizing and tailoring management strategies for each breed. The objective of this study was to evaluate the association of breed with key growth performance indicators, mortality, and morbidity of common health disorders such as diarrhea, bovine respiratory disease (BRD), and abomasal bloat. A total of 120 1-d-old calves (54 Holsteins and 66 B $\times$ D) were housed individually in outdoor hutches and fed 6 L of milk replacer daily. Calves were offered water and texturized calf starter ad libitum for the duration of the study; the amount offered each day was weighed and subtracted from the leftovers, which were also weighed the following day, to calculate the initial daily intake of calves. Body weight was measured at arrival and at d 15 and 56 of age. Calves were visually inspected daily for 56 d, with fecal consistency scored on a 4-point scale and a systematic scoring system used to assess BRD. Mixed linear regression models and Cox proportional hazard models were fitted to the data, with breed, body weight at arrival, failure of passive transfer, and calf sex as independent variables. Breed was not associated with the incidence of diarrhea ($P = 0.45$), BRD ($P = 0.28$), abomasal bloat ($P = 0.09$), or mortality ($P = 0.20$). Breed was associated with average daily gain within the first 56 d of life, as B $\times$ D gained 0.09 kg/d (SE: 0.02) more than Holsteins ($P < 0.01$). This is likely because B $\times$ D calves consumed more calf starter and at a younger age than Holsteins. The time to consume 100 g/d of starter intake for 2 consecutive days was 4 d shorter for B $\times$ D calves than Holsteins ($P < 0.01$), and they ate 0.34 kg/d (SE: 0.05) more calf starter during the preweaning period than Holsteins ($P < 0.01$). These results suggest that morbidity and mortality are similar between B $\times$ D and Holsteins, but growth performance is greater for B $\times$ D calves, likely due to increased calf starter intake during the preweaning period.

Key Words: beef-on-dairy, average daily gain, health

2004 Effect of trace mineral supplement form on mastitis outcomes. K. R. Montgomery*, A. E. Relling¹, M. X. S. Oliveira¹, K. M. Enger¹, S. Wellert¹, J. E. Coplin¹, H. L. M. Tucker², and B. D. Enger¹, ¹The Ohio State University, Wooster, OH, ²NOVUS International Inc., Chesterfield, MO.

Mastitis negatively affects milk composition, partly by damaged secretory epithelium. Trace minerals are cofactors in a myriad of processes related to oxidative balance, immune responses, and epithelial barrier function, but trace mineral absorption is affected by supplement form. This study evaluated milk composition and indicators of epithelial damage in cows supplemented with sulfate or methionine hydroxy analog chelate (MMHAC) of Zn, Cu, and Mn at isometal levels during

sterile mastitis. Midlactation multiparous Holstein cows ($n = 36$) were blocked by DIM and milk yield and received either (1) sulfate trace minerals and intramammary infusions of saline (SULF-CON, $n = 12$), (2) sulfate trace minerals and intramammary infusions of killed *Staphylococcus aureus* (SULF-SA, $n = 12$), or (3) MMHAC trace minerals and intramammary infusions of killed *Staph. aureus* (MMHAC-SA, $n = 12$). The study lasted 6 wk, and cows were repeatedly intramammarily infused every 3 d during the final 2 wk to elicit chronic mastitis. Milk composition was assessed during the final challenge. Data were analyzed using PROC GLIMMIX in SAS; means were compared using LSMeans. Fixed effects included treatment, time, and their interaction; block and cow within block were random effects. Both MMHAC-SA and SULF-SA SCS were similar ($P = 0.7$) but greater than SULF-CON ($P < 0.01$). Milk lactose content differed by treatment ($P < 0.01$). The SULF-CON treatment had greater lactose content than SULF-SA ($P < 0.01$), whereas MMHAC-SA lactose content was intermediate, being slightly less than that of SULF-CON ($P = 0.08$) and slightly greater than SULF-SA ($P = 0.08$). Milk Na contents of MMHAC-SA and SULF-SA were similar ($P = 0.4$) and were marginally or significantly greater than SULF-CON ($P = 0.07$ and $P = 0.02$, respectively). Milk Fe content of MMHAC-SA and SULF-SA did not differ from SULF-CON ($P \geq 0.23$), but Fe content of MMHAC-SA was less than SULF-SA ($P = 0.02$), suggesting the potential for reduced leakage of blood into milk. Results indicate that repeated insults of killed *Staph. aureus* negatively affect milk composition, and that trace mineral supplement form may differentially affect some markers of mammary epithelial barrier integrity during a chronic mastitis event.

Key Words: chelate, chronic mastitis, epithelium

2005 Housing, husbandry, and health management practices on Quebec dairy farms. H. Jim*, E. Ribeiro¹, B. Mion², T. F. Duffield¹, and R. Couto Serrenho¹, ¹University of Guelph, Guelph, ON, Canada, ²Lactanet, Sainte-Anne-de-Bellevue, QC, Canada.

Best management practices (BMP) for commercial dairy farms are widely available, yet there is limited understanding of their implementation and the challenges producers face in achieving these standards. The objective of this retrospective descriptive study was to describe Quebec dairy herds' housing and management practices. Pre-existent survey data (housing, husbandry, and herd health; 35 questions) collected by Lactanet ($n = 116$; Mar. 2020–Feb. 2021) from 1,965 herds and respective DHI data were used. Results from freestall herds (FS; 20%) were segregated from tiestall herds (TS; 80%) and summarized using descriptive statistics. The average herd size was 76 ± 56 cows, with 65 ± 47 milking cows, peaking at 40 ± 5 kg/d. Of FS, 54% milked cows on automated milking systems, and of TS, 98% used a pipeline system. Straw was the most common bedding in FS and TS (>40%), with TS primarily bedding $\geq 2 \times/d$ (>55%), and FS bedding maternity area $\geq 2 \times/d$ (25%) and other cow groups weekly (27%). Most cow groups in TS lacked pasture and exercise pens (>65%), except that 70% of TS far-off groups had pasture access. Most FS and TS (>85%) maintained daylight intensity >200 lx, with building lights on for 14 to 17 h/d. In FS, headlock feed bunk gates were most common (>45%), except for lactating cows, where horizontal bars predominated (40%). Calvings mostly occurred in pens (83%) and tiestalls (58%) for FS and TS, respectively. Over 80% of FS and TS dried cows using a single protocol, regardless of production level. In FS and TS, most herds mentioned reducing feed (>59% and >75% respectively) and using intramammary antibiotic (IMM) without teat sealant (45%); 41% of TS mentioned using IMM with sealants. Although >50% of FS used footbaths, >75% of TS used feet sprays; overall, hoof trimming was conducted $\geq 2 \times/yr$ (>80%). This

study described adult cow management practices on Quebec farms. The research team identified as strengths lighting, dry-off feed reduction, hoof trimming, and TS bedding management; and as potential improvement points, production level customized dry-off protocol and use of antibiotic with teat sealant, bedding frequency and feed bunk gate for lactating cows in FS, and exercise pen access and footbath use in TS.

Key Words: milking cow, welfare, survey

2006 Does hay improve performance in pair-housed dairy calves? G. D. Plaughner*, I. L. B. Fernandes, B. J. Bone, and M. C. Cantor, *The Pennsylvania State University, University Park, PA.*

The objective of this randomized control trial was to evaluate the effects of feeding pelleted hay to pair-housed dairy calves (2 calves per pen) on BW, ADG, calf starter DMI, and feed efficiency (FE). Holstein heifer calves ($n = 32$ pairs; 64 calves) were enrolled at pairing (5 ± 3 d of age; mean $\pm$ SD) and randomly assigned in blocks of 4 to a control (CON; no hay) or treatment (HAY; pelleted timothy). All calves received 1.0 kg/d of milk solids reconstituted to 7.41 L/d (135 g/L; 22% CP, 20% fat) until d 56 and 3.2 L/d from d 57 to 62, and were weaned on d 63. The HAY group had a trough with pellets until d 59 and then were transitioned to loose timothy hay until study completion on d 70 (7 d postweaning). Starter and hay intake and orts were recorded daily. We measured BW twice weekly until 30 d and then weekly, with a final BW on d 70. We calculated the average BW, ADG, starter DMI, and FE by period, where period 1 = birth to d 21, period 2 = d 22 to 55, period 3 = d 56 to 62, and period 4 = d 63 to 70. The measurement unit was pair. We used mixed linear regression models to assess the effects of HAY, period, and the HAY $\times$ period interaction on BW, ADG, starter DMI, and FE. We adjusted for birth weight and serum total protein, with period as a repeated measure and pair as the subject, and block as a random effect. We used Tukey adjustments to correct for multiple mean comparisons. Both HAY and period were associated with BW ($P < 0.001$). There was a tendency for a HAY $\times$ period interaction for BW ($P = 0.06$), where HAY pairs grew better in periods 2, 3, and 4 compared with controls ($P \leq 0.02$). Final BW on d 70 were significantly different (CON 95.7 ± 4.33 kg vs. HAY 102.5 ± 6.31 kg; $P = 0.001$). There was no association of HAY with ADG (CON 0.85 ± 0.04 kg/d vs. HAY 0.94 ± 0.04 kg/d; $P > 0.05$), starter DMI (CON 2.68 ± 0.15 kg vs. HAY 2.50 ± 0.14 kg; $P > 0.05$), or FE (CON 0.65 ± 0.07 vs. HAY 0.73 ± 0.07 ; $P > 0.05$). We

suggest that pelleted timothy hay can be offered to pair-housed calves to improve BW gains without compromising calf starter intakes.

Key Words: hay, average daily gain, social housing

2007 Effects of α -amylase-enhanced corn silage on digestibility, milk yield, and milk composition: A meta-analysis. A. D. Majewski* and K. C. Krogstad, *The Ohio State University, Columbus, OH.*

Corn silage enhanced with α -amylase increases α -amylase activity within the corn kernel. This enzyme has the potential to aid in the breakdown of starch, increasing starch digestibility and the sugars made available from the corn grain. Our meta-analysis investigated the effects of feeding diets containing α -amylase enhanced corn silage (ENO), compared with a control corn silage (CON), to lactating dairy cows. The objective was to observe how ENO affected the dry matter intake, digestibility, milk yield, and milk component yields from lactating dairy cows. We hypothesized that ENO would increase dry matter digestibility, feed efficiency, and milk yield. Following a systematic review of literature, data for this meta-analysis were extracted from 4 peer-reviewed studies and analyzed using the “metafor” package (R, version 4.4.1) with a random effects model. The cows in these studies ranged from 79 to 170 DIM, consumed 25.1 to 28.0 kg/d of dry matter, and made 34.5 to 42.7 kg/d of ECM. We found that ENO tended to increase total-tract CP digestibility compared with CON (1.11 ± 0.47 , $P = 0.10$). The total-tract digestibilities of starch, NDF, and DM did not differ between treatments ($P = 0.21$, $P = 0.16$, and $P = 0.26$, respectively). Milk yield was similar between ENO and CON (0.10 ± 0.75 , $P = 0.90$), but MUN was reduced in cows fed ENO compared with CON (-0.72 ± 0.21 , $P = 0.03$). Milk fat and protein percentages did not differ between treatments ($P = 0.46$, $P = 0.17$, respectively). Despite the limited effects that feeding ENO had on milk production, the numerically increased total-tract digestibilities of NDF and DM, in addition to the tendency to increase total-tract digestibility of CP, suggest a more complete digestion of feed, which may result in increased microbial protein synthesis, improved rumen ammonia use, and reduced MUN. One limitation of this analysis is that it used a small database. As additional peer-reviewed data become available, these will be added to this meta-analysis.

Key Words: forage, digestibility, corn silage, α -amylase, Enogen

ADSA-GSD Competition: Production (PhD) Poster Semifinalists

2008 Alterations to lung function, immune system, and productivity of dairy cows exposed to wildfire PM_{2.5}. M. Kamyabi^{*1}, D. Sarantopoulos¹, A. Pace¹, L. De Moura Pereira¹, D. Konetchy¹, P. Rezamand^{1,2}, and A. L. Skibieli¹, ¹University of Idaho, Moscow, ID, ²University of Connecticut, Storrs, CT.

Fine particulate matter (PM_{2.5}) in wildfire smoke poses health risks. We investigated cow pulmonary and immune function during and after wildfire PM_{2.5} exposure to understand the pathophysiology of wildfire PM_{2.5} inhalation. Lactating Holstein cows (n = 21) were monitored across the 2024 wildfire season. Daily average temperature-humidity index (THI) and PM_{2.5} concentrations were calculated. Daily milk yields (MY) were recorded. At 3 time points, blood and milk were collected, and lung function (tidal volume [VT], minute ventilation [VE], and respiratory rate [RR]) and rectal temperature (RT) were measured. The first 2 samplings (WF1, WF2) each occurred 1 d after 72-h episodes of elevated wildfire PM_{2.5} (concentrations >25 µg/m³), with 1 mo between WF1 and WF2. The third sampling (POST) was 2 wk after WF2, and the previous 72-h PM_{2.5} concentration was 5 µg/m³. Blood hematology, granulocyte phagocytosis, and acute-phase proteins were measured. Milk fat and protein (%) and SCC were quantified. Data for MY were analyzed with separate mixed models to assess lags (delayed responses) up to 7 d, with fixed effects of PM_{2.5}, THI, and interactions. All other data were analyzed using mixed models with fixed effects of day (WF1, WF2, POST), parity (1 or >1), and interactions. All models included a repeated effect of day within cow and random effect of cow. During WF1 and WF2, VT was decreased, but RR was increased compared with that of POST ($P < 0.03$). Eosinophils and basophils increased in WF2 and remained elevated at POST ($P < 0.04$). Monocytes increased during WF2 but declined at POST ($P < 0.04$). Phagocytosing granulocytes (%) were greater at WF1 than POST ($P = 0.0002$). The POST serum amyloid A was higher than WF2 ($P = 0.01$). Elevated PM_{2.5} × THI decreased MY on lag d 6 and 7 ($P < 0.04$). Milk fat percentage was lower at WF2 and POST relative to the WF1 ($P < 0.001$). Milk protein percentage was lowest at WF1 in multiparous cows ($P = 0.007$). Compared with WF1, primiparous cows had lower SCC at WF2. Multiparous cows had higher SCC at POST ($P < 0.04$). These findings suggest that wildfire PM_{2.5} inhalation can alter pulmonary function and systemic health, affecting dairy cow productivity.

Key Words: air pollution, ventilation, inflammation

2009 Genetic evaluation of novel estrus behavior traits in Holstein cows using activity-monitoring collars. G. R. Dodd^{*1}, F. Miglior², F. S. Schenkel¹, and C. F. Baes¹, ¹Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, ²Lactanet Canada, Guelph, ON, Canada, ³Applied Animal Biology, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada.

Fertility is a crucial component for dairy farm productivity but remains a major challenge due to the complex interplay of reproductive and environmental factors. Recent advances in estrus detection technology, such as the use of activity-monitoring collars, can provide valuable insights into key fertility indicator traits, including maximum intensity of estrus behavior (MEI), estrus duration (ED), and the interval from calving to first estrus activity (CTFE). These traits are biologically relevant indicators of fertility, as they are associated with improved pregnancy and ovulation rates and enhanced embryonic survival. The aim of this

study was to estimate the genetic parameters of MEI, ED, and CTFE in lactating Holstein cows and to assess the reliability of estimated breeding values (EBVs) for these traits. Data were collected using activity-monitoring collars (CowScout, GEA) on a single Slovakian farm from January 2020 to March 2023. The data set included 8,580, 8,868, and 3,034 records from 2,403, 2,432, and 2,207 cows for MEI, ED, and CTFE, respectively. Repeatability animal models were used for each trait, accounting for permanent environmental effects across lactations for all 3 traits, as well as for within-lactation permanent environmental effects for MEI and ED. Heritability estimates were 0.08 ± 0.03 for MEI, 0.14 ± 0.01 for ED, and 0.08 ± 0.03 for CTFE. Repeatability across lactations was estimated at 0.17 ± 0.02 for MEI, 0.18 ± 0.01 for ED, and 0.33 ± 0.03 for CTFE. Repeatability within lactation was 0.15 ± 0.03 for MEI and 0.14 ± 0.01 for ED. The average reliability of EBVs of phenotyped cows were low to moderate, at 0.34 ± 0.08 for MEI, 0.46 ± 0.09 for ED, and 0.24 ± 0.06 for CTFE. For proven sires, reliability was moderate to high, reaching 0.67 ± 0.15 for MEI, 0.78 ± 0.13 for ED, and 0.48 ± 0.14 for CTFE. This study demonstrates the potential for incorporating novel estrus behavior traits into breeding programs to improve fertility in dairy cattle.

Key Words: fertility indicator traits, estrus behavior, genetic selection

2010 Genetic parameter estimation for first service to conception: A potential female fertility trait in US dairy cattle evaluations. G. Dutta^{*1}, T. McWhorter², A. Legarra², J. Graham³, J. B. Cole^{2,4}, J. Burchard², and B. Fragomeni¹, ¹University of Connecticut, Storrs, CT, ²Council on Dairy Cattle Breeding, Bowie, MD, ³USDA-AGIL, Beltsville, MD, ⁴Department of Animal Sciences, Donald Henry Barron Reproductive and Perinatal Biology Research Program, and the Genetics Institute, University of Florida, Gainesville, FL.

The Council on Dairy Cattle Breeding (CDCB) currently provides evaluations for 4 female fertility traits in US dairy cattle: daughter pregnancy rate (DPR), cow conception rate (CCR), heifer conception rate, and early first calving. Evolving reproductive management practices, such as extended voluntary waiting periods (VWP) for high-producing and first-parity cows, present new challenges. The current DPR calculation assumes a standardized VWP of 50 d + 20 d for all cows. The FSC is calculated as the number of days between first service and conception (linear [L]-FSC). Additionally, a nonlinear transformation of the trait was tested (NL-FSC). Regardless of when the cow was first inseminated, FSC measures the time it took her to conceive after the attempts began. This makes FSC independent of VWP, providing greater flexibility in fertility reporting and evaluation. This study aims to estimate the genetic parameters for FSC using data from US dairy herds. Dairy animals that calved between 2018 and 2022 were retrieved from the National Collaborator Database, comprising 95.65 million phenotypic records. Five random subsamples were generated by sampling 100,000 unique animal identification numbers. In each subsample, the number of cow management groups (herd-year-season-parity-registry status code) was capped at 10,000. Cow management groups with fewer than 4 observations were removed. The pedigree file contained 51 million entries tracing back 3 generations. Variance components were estimated using a repeatability animal model. The average heritability for NL-FSC, including random herd by sire effect across subsamples, was 0.006 ± 0.03 . For a similar model, heritability for L-FSC, including random herd by sire effect, was 0.016 ± 0.02 . Because of slow convergence, the random herd by sire effect was removed from the L-FSC model, resulting in a heritability of

0.019 ± 0.01. The next step involves estimating variance components for DPR and CCR, followed by genetic correlations between these traits and FSC, to assess their utility in multitrait genetic evaluations. Preliminary results suggest that FSC could serve as a valuable indicator of fertility, complementing current models in genetic evaluations in the future.

Key Words: voluntary waiting period (VWP), first service to conception interval (FSC), fertility

2011 Investigation of the expression of candidate genes associated with a twinning rate QTL located on bovine chromosome 11. M. Bakherad^{*1}, J. P. Nascimento Andrade², S. Molaei Moghbeli¹, J. Gille¹, M. C. Wiltbank¹, and B. W. Kirkpatrick¹, ¹*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI*, ²*West Texas A&M University, Canyon, TX*.

Twin births in dairy cattle present challenges for producers, leading to health issues for both cows and calves that impact profitability. Studies using GWAS analyses of twinning rates in Holstein cattle have reported the most significant genomic association with twinning rate in a region containing 2 strong candidate genes, follicle-stimulating hormone receptor (*FSHR*) and luteinizing hormone chorionic gonadotropin receptor (*LHCGR*). Genetic variants in the coding sequence of these genes were not associated with twinning rate, leading to the hypothesis that one of the two genes is differentially expressed in association with twinning rate. Granulosa cells were collected from 30 Holstein cows undergoing estrus synchronization using the double-ovsynch protocol. Cows were chosen to provide equal representation of genotypes for the twinning rate QTL based on the most significantly associated SNP. Five days following timed artificial insemination cows received a 100-μg dose of gonadotropin-releasing hormone to initiate a new follicular wave, and 7 d later granulosa cells were aspirated from the largest follicle. RNA was extracted from the granulosa cells, and gene expression was assessed by real-time quantitative PCR with data analyzed by the delta-delta Ct method. Association of gene expression with QTL genotype was tested by simple linear regression of linear-transformed delta Ct values on count of the SNP G allele associated with increased twinning rate. The QTL genotype was significantly associated ($P < 0.005$) with expression of *FSHR* but was not associated with *LHCGR* expression. Increased *FSHR* expression was associated with increasing copy number of the G allele and thus increased twinning rate.

Key Words: *FSHR*, *LHCGR*, granulosa cells

2012 Characterization of dry-period mammary acetate and glucose metabolism and their association with colostrum production in multiparous Holstein cattle. A. J. Fischer-Thustos^{*}, K. Klein, J. Petrou, J. Cant, and M. A. Steele, *Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada*.

Mammary metabolism increases during the dry period to prepare for the subsequent lactation; yet the time course of mammary metabolic activity and its relationship with colostrum production require further investigation. Thus, this study aimed to characterize mammary acetate and glucose (glc) metabolism during the dry and calving periods and to determine potential associations with colostrum production. Holstein cows ($n = 11$ second-parity cows; $n = 10$ third-parity cows) were dried off at $d -58 \pm 2.9$ before calving. Mammary acetate and glc arteriovenous difference and uptake were determined biweekly until 1 wk before expected calving, then every 4 d until actual calving, and at calving by collection of pairs of tail and milk vein serum samples and estimation of mammary blood flow via Doppler ultrasonography of the milk vein.

Serum acetate and glc were analyzed by spectrophotometric enzymatic assays. Colostrum was collected within 1.4 ± 0.76 h of calving and components determined via mid-infrared spectroscopy. Data were analyzed using PROC GLIMMIX of SAS, considering the fixed effects of parity and time and the random effect of cow. Mammary acetate uptake remained constant ($P > 0.24$) until steadily increasing ($P < 0.01$) from wk -1 until calving, and mammary glc uptake only increased ($P < 0.01$) at the onset of calving. Regression analysis demonstrated that previous lactation peak milk yield (linear term: -0.29; partial $R^2 = 0.36$; $P < 0.01$) and far-off mammary acetate uptake (linear term: -0.14; partial $R^2 = 0.10$; $P = 0.06$) negatively impacted colostrum yield. Correlation analyses demonstrated similar relationships ($r = -0.54$ to -0.70) between colostrum production and far-off mammary acetate uptake but no relationship ($r < 0.33$) with close-up mammary metabolism. This work demonstrates that acetate may be of greater importance in mammary redevelopment than glc. In addition, the relationships presented herein suggest that achieving early dry-off may be a more important determinant of colostrum production than high mammary metabolic activity during the close-up period.

Key Words: dry period, mammary metabolism, colostrum

2013 The effects of saturated fatty acid (FA) supplements and a high-UFA diet that caused milk fat depression on plasma concentration of fatty acid esters of hydroxy fatty acids (FAHFA) in dairy cows. M. Arif^{*}, B. A. Harsch, C. Matamoros, R. Shepardson, and K. J. Harvatine, *Pennsylvania State University, University Park, PA*.

Fatty acid esters of hydroxy fatty acids (FAHFA) are a novel class of bioactive lipids synthesized by esterification of fatty acid (FA) with hydroxy fatty acid (HFA). The objective of this study was to characterize the concentration of FAHFA in plasma of dairy cows fed different saturated FA supplements and a high-UFA diet that caused milk fat depression (MFD). We hypothesized that these changes would impact the concentrations of FAHFA in blood of lactating cows. Plasma samples were analyzed from 2 previous experiments. Experiment 1 compared a low-fat control diet (CON) to diets that contained 1.95% of a fat supplement high in palmitic acid (PA), high stearic acid (SA), or a blend of PA and SA using 12 multiparous Holstein cows in a 4×4 Latin square design with 21-d periods. Experiment 2 compared a low-fat control diet to a diet that contained a high-PA supplement at 2% of DM and a lower-fiber and higher-starch diet that contained 2.2% soybean oil and used 12 Holstein cows in a 3×3 Latin square design with 21-d periods. The concentrations of FAHFA in plasma were quantified using liquid chromatography-tandem mass spectrometry. Data were analyzed using mixed models that included treatment as a fixed effect and cow and period as random effects, and repeated measures were used in experiment 2 as concentration was measured at 0500 h and 1700 h. Five FAHFA were identified and quantified in experiment 1 and 6 in experiment 2, and all were affected by treatment ($P < 0.05$). In experiment 1, the concentrations of 9-palmitic acid hydroxy palmitic acid (PAHPA) and 9-stearic acid hydroxy stearic acid (SAHSA) were highest in PA and SA, respectively, whereas 5-palmitic acid hydroxy stearic acid (PAHSA), 9-PAHSA, and 10-PAHSA were highest with PA+SA. In experiment 2, 9-PAHPA, 5-PAHSA, 9-PAHSA, 10-PAHSA, and 11/12 PAHSA were increased with PA. The concentrations of 10-PAHSA and 9-SAHPA were decreased with MFD. Overall, PA and SA supplements and MFD affected the concentration of FAHFA in plasma, demonstrating a direct effect of dietary FA on this emerging regulator of metabolism. Functional roles for these lipids will require further exploration.

Key Words: fatty acid esters of hydroxy fatty acids (FAHFA), milk fat depression (MFD), palmitic acid

2014 Risk factors for retained placenta in dairy cows from tropical herds. K. R. Oliveira^{*1,2}, T. E. da Silva², J. H. C. Costa², T. M. Ferreira³, C. E. C. Consentin⁴, R. Sartori⁵, A. L. Silva¹, and P. P. Rotta¹, ¹*Universidade Federal de Vicosa, Vicosa, MG, Brazil*, ²*University of Vermont, Burlington, VT*, ³*Alta Genetics do Brasil, Uberaba, MG, Brazil*, ⁴*GlobalGen Vet Science, Jaboticabal, SP, Brazil*, ⁵*ESALQ, University of São Paulo, Piracicaba, SP, Brazil*.

Retained placenta (RP) is the failure to expel fetal membranes within 24 h after birth, influenced by environmental conditions, seasonality, calving complications (e.g., twins, abortion), and maternal age. High-producing cows face increased RP risk due to genetic and environmental factors. This study investigates RP risk factors in tropical dairy herds. Data from 630 Brazilian farms were collected from the “Concept Plus Dairy” program (Alta Genetics do Brasil) from January 2021 to December 2023 in 10 states. A technician survey assessed production systems: compost barn, freestall, and semi-intensive. After data cleaning, 306 farms and 97,035 calvings from Holstein (57.4%) and Holstein × Gyr cows (1/2, 3/4, 7/8) were analyzed. A binomial model in “glmmTMB” considered RP as the outcome variable. Fixed effects included lactation number (1 to 7), twin pregnancies, calving season, calf sex, calving type (normal, assisted, stillbirth, premature, abortion), production system, farm size, breed, milk yield, and calving year, with farm as a random effect. Variables were selected via Akaike information criterion using “buildglmmTMB.” Adjusted marginal means were estimated using “emmeans,” with pairwise comparisons via “cld” ($\alpha = 0.05$). Mean RP incidence was $27.5\% \pm 1.64\%$, higher than in subtropical (Canada, United States) and tropical (Iran) systems. Probability of RP increased with parity, from $24.0\% \pm 1.53\%$ (first calving) to $42.5\% \pm 2.18\%$ (seventh). Twin pregnancies raised RP risk from $16.1\% \pm 1.08\%$ to $43.0\% \pm 2.20\%$. Male calves slightly increased RP risk ($29.3\% \pm 1.73\%$) vs. females ($25.8\% \pm 1.59\%$). Premature calvings and abortions had higher RP risks ($47.8\% \pm 2.70\%$ and $47.3\% \pm 5.21\%$) than normal calvings ($10.5\% \pm 0.66\%$). Holsteins had higher RP incidence ($29.3\% \pm 1.70\%$) than Holstein × Gyr cows ($25.7\% \pm 1.89\%$). The RP risk peaked in 2021 ($28.6\% \pm 1.72\%$), declined in 2022 ($26.7\% \pm 1.63\%$), and stabilized in 2023 ($27.3\% \pm 1.70\%$). Farm size, production system, and milk yield did not directly affect RP but may interact with breed effects. Parity, twin pregnancies, calving type, and breed influence RP risk in tropical dairy herds, requiring targeted management to reduce its incidence.

Key Words: tropical dairy system, crossbreed cow

2015 Effect of incremental levels of synthetic biochanin A on production performance and ruminal fermentation in dairy cows. M. A. Arshad^{*}, M. L. Fischer, M. A. Rahman, W. Sims, O. R. Osualale, K. V. Almeida, and A. F. Brito, *Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, NH*.

Biochanin A (BCA) is an isoflavone phytoestrogen commonly found in red clover (*Trifolium pratense* L.), and it has been shown to inhibit the hyper ammonia-producing bacteria in the rumen and improve N use efficiency in dairy cows. We aimed to investigate the effect of incremental dietary levels of synthetic BCA on DMI, milk yield (MY) and composition, and ruminal fermentation in dairy cows. Twelve multiparous (105 ± 31 DIM and 722 ± 50 kg BW) and 3 primiparous (92 ± 5 DIM and 572 ± 24 kg BW) Holstein cows were blocked by MY, DIM, and parity, and, within block, randomly assigned to treatment sequences in a replicated 3×3 Latin square design. Each period lasted 28 d, with 21 d for diet adaptation and 7 d for data and sample collection. Treatments were (1) control diet without BCA (CON), (2) CON plus 14 g/d of synthetic BCA (L-BCA), or (3) CON plus 28 g/d of

synthetic BCA (H-BCA). Data were analyzed in SAS using orthogonal polynomial contrasts (linear and quadratic). Ruminal fluid was collected via stomach tubing 4 h after the a.m. and p.m. feedings for 2 consecutive days. Neither DMI (mean = 25.7 kg/d) nor MY (mean = 41.7 kg/d) were affected by diet. However, yields of 4% FCM (41.9, 40.8, and 40.2 kg/d) and ECM (42.9, 41.7, and 41.1 kg/d) tended to reduce linearly ($P = 0.07$) with feeding CON, L-BCA, and H-BCA, respectively. We also observed linear reductions ($P < 0.02$) in milk lactose concentration (4.82%, 4.83%, and 4.86%) and yield (2.07, 1.99, and 1.95 kg/d) in cows fed CON, L-BCA, and H-BCA, respectively. Feed efficiency, concentration and yield of milk fat, milk true protein concentration, and MUN were unaffected by treatments. Contrarily, milk true protein yield (1.43, 1.38, and 1.37 kg/d) tended to decrease linearly ($P = 0.08$) in CON, L-BCA, and H-BCA, respectively. Treatments did not change ruminal pH (mean = 6.31), ruminal concentrations of total VFA (mean = 100 mM), or $\text{NH}_3\text{-N}$ (mean = 4.99 mM), nor the ruminal molar proportions of acetate (mean = 63.4%), propionate (mean = 24.9%), or butyrate (mean = 9.45%). In brief, feeding BCA did not improve MY and N utilization in dairy cows.

Key Words: biochanin A, milk production, rumen fermentation

2016 Assessing the relative bioavailability of rumen-protected methionine using the selenomethionine and plasma free methionine technique. J. Kim^{*1,2}, H. Hu², K. Park^{1,2}, and C. Lee^{1,2}, ¹*Interdisciplinary Nutrition Program, Department of Animal Sciences, The Ohio State University, Wooster, OH*, ²*Department of Animal Sciences, The Ohio State University, Wooster, OH*.

The objective of the study was to compare the relative bioavailability (BA) of commercial rumen-protected Met (RP-Met) products as determined by the selenomethionine (Se-Met) and plasma free methionine technique (PFM). A total of 36 mid-lactating Holstein cows were used. All cows received a basal diet for 10 d as diet adaptation, followed by the backgrounding diet (basal diet containing 0.15% Se-yeast) for the subsequent 14 d. After the backgrounding period, cows received the experimental diets in a replicated 4×4 Latin square with 14-d periods. The experimental diets were the backgrounding diet supplemented with 1 of 4 commercially available RP-Met products (RP-Met A, B, C, and D). The diets were delivered to cows once daily at 0800 h. Milk samples were collected from 4 consecutive milkings on the last 2 d in each period. Milk samples were analyzed for total N and Se, and the relative BA was determined according to the change of the ratio of Se to N between the backgrounding and experimental period (Se-Met). Blood samples were collected from the coccygeal vein at 3 and 6 h after feeding on the last 2 d in each period. The change of plasma Met as percentage of total amino acid between the backgrounding and experimental period was used to calculate the relative BA (PFM). The statistical analysis was conducted using the Mixed procedure of SAS with random effects of block, cow within block, and period and a fixed effect of treatment. Dry matter, Se, and Met intake did not differ among treatments. Milk Se/N tended to differ ($P = 0.07$) and plasma Met as percentage of total amino acid was different ($P < 0.01$) among treatments, indicating that the BA was different among the RP-Met products. The relative BA calculated via Se-Met and PFM were 78.5% and 69.9% for RP-Met A, 55.2% and 36.2% for RP-Met B, and 83.7% and 87.7% for RP-Met C, respectively, assuming RP-Met D is 80% bioavailable. In conclusion, there was a good agreement on the ranking ($C > D > A > B$) and a reasonable agreement on the relative BA of the RP-Met products between Se-Met and PFM.

Key Words: milk selenium, plasma amino acid, Se-yeast

Animal Behavior and Well-Being 1: Posters

2017 A scoping review of research validating precision wearable sensors for monitoring dairy cattle behavior. A. M. Lee^{*1}, M. Brause², D. Foy², and M. C. Cantor¹, ¹*Pennsylvania State University, University Park, PA*, ²*AgriGates LLC, Philadelphia, PA*.

For our scoping review, we identified the validity of precision livestock farming (PLF) validation work by (1) assessing the common statistical criteria used to validate wearables for dairy cattle behavior. We also (2) set validity criteria and compared the literature to our standards. We used the PICO method (population, intervention, comparison, and outcome) and asked, "How do we define the need for validation standards when considering precision technologies to record dairy cattle behavior?" Key words were extracted from PubMed and Web of Science, and 2,955 articles were collected. After a validated sifting process, we extracted 101 articles. We assessed the literature using Power BI (Microsoft Corp.) to extract the precision, accuracy, and bias (Bland-Altman plots, deviations from mean, best fit line, bias correction factors, or location scale shift). We also used Power BI to confirm the sensitivity, specificity, reproducibility (sample size, sensor type), and comparison method. One researcher manually categorized dairy cattle behavior into 10 categories for consistency. We considered a PLF wearable study to have validity if they reported a high precision $\geq 90\%$, did not observe bias, used reproducibility parameters, used a comparison method, and used an ethogram to describe the behavior. One researcher manually reviewed all papers that were reported as lacking bias measurements by Power BI, because multiple statistical techniques were used to assess bias. We found a very high reproducibility 93% (94/101) among studies. Activity behavior (61/101), feeding behavior (59/101), and resting (55/101) were commonly validated with wearable sensors in dairy cattle. However, precision was rarely calculated 40% (40/101) among studies. For studies that reported precision, 90% (36/40) met our criteria for precision and reproducibility. However, we observed that many precise studies failed to report and/or observe a lack of bias 35% (14/40). Thus, a negligible level of research met our validity criteria, 14% (14/101). We suggest that PLF validation studies for dairy cattle management need to meet precision $\geq 90\%$, observe a lack of bias, and be reproducible for validity.

Key Words: precision livestock farming, technology

2018 Comparing behavioral responses to heat stress and reticulorumen temperature to 2 different heat mitigation systems in a commercial dairy farm. L. Morciglio-Matías^{*1}, J. Hollist², V. Narayanan², and C. B. Tucker¹, ¹*Center for Animal Welfare, Department of Animal Science, University of California Davis, Davis, CA*, ²*University of California Davis Western Cooling Efficiency Center, Davis, CA*.

Heat mitigation strategies typically combine shade, ventilation, and soakers to reduce the adverse effects of heat stress. The goal of this study was to compare responses associated with heat stress in dairy cattle across 2 heat mitigation systems using fans and soakers in freestall barns. The first system was a conventional baseline controller (Baseline), which adjusted cooling based on set environmental temperature thresholds determined by the farm, and the second used an optimized algorithm that tailored cooling frequency to maintain desired, modeled core temperature (Optimized). Holstein cows ($n = 16$; DIM = 64.3 ± 16.7 ; lactation number = 2.4 ± 0.7), housed in a single pen on a commercial dairy farm in Merced County, California, were selected as focal animals. Data were collected via in-person scan sampling at 45-min intervals between 1000 h and 1900 h over a 12-d period (8 d and 4 d,

Baseline and Optimized, respectively) in summer. Observed behaviors included respiration rate and panting score (assessed by the presence or absence of drool and open-mouth panting). Internal temperatures were recorded every 10 min using reticular boluses (smaXtec, Graz, Austria) for each focal animal. Average values were compared with paired *t*-tests. Reticulorumen temperature between 1000 and 1900 h was lower in the Optimized condition, compared with Baseline (39.76 vs. $39.92 \pm 0.03^\circ\text{C}$, respectively, $P < 0.01$). Respiration rates indicated that cattle were appropriately cooled and did not differ between controllers (Optimized vs. Baseline: 62 vs. 63 ± 2 breaths/min). Similarly, drooling and open-mouth panting were rare and similar across treatments (drool: 2.4 vs. $3.0 \pm 0.9\%$ observations, $P = 0.36$; open-mouth panting: 0.8 vs. $1.2 \pm 0.5\%$ observations, $P = 0.28$; Optimized vs. Baseline, respectively). Compared with a conventional controller, the optimized algorithm performed well, keeping body temperature lower, although both systems worked well, as indicated by respiration rates, drooling, and open-mouth panting. Our next steps are to replicate this study on 3 other commercial sites.

Key Words: heat stress behavior, heat mitigation system

2019 Changes in activity related to mastitis in organic dairy cows. J. Bonney-King¹, E. E. Lindner¹, V. E. Cabrera², B. J. Heins³, R. A. Lynch⁴, G. M. Schuenemann⁵, E. Silva², J. Velez⁶, P. Pinedo⁷, A. De Vries¹, and E. K. Miller-Cushon^{*1}, ¹*University of Florida, Gainesville, FL*, ²*University of Wisconsin, Madison, WI*, ³*University of Minnesota, Morris, MN*, ⁴*Cornell University, Ithaca, NY*, ⁵*The Ohio State University, Columbus, OH*, ⁶*Aurora Organic Farms, Platteville, CO*, ⁷*Colorado State University, Fort Collins, CO*.

This study assessed changes in activity as potential indicators of mastitis in organic dairy cows. Using data collected on a certified organic dairy farm, we analyzed daily behavioral activity measurements (standing time, standing bout frequency, step count) obtained using leg-based accelerometers (iceQube, IceRobotics) on cows ($n = 634$) with a focus on the period surrounding the diagnosis of clinical mastitis ($d 0 \pm 30$ d). Cows were housed in sand-bedded freestall barns with continuous access to dry lots, with access to pasture during the warmer months (≥ 8 h/d; Apr.–Sep.). Upon diagnosis, cows were moved to a hospital pen and, in severe cases, administered aspirin and electrolytes. Data were analyzed using generalized linear mixed models, including days relative to diagnosis with mastitis as a repeated measure, with fixed effects of season and concurrent health events (diagnosed within the same week), parity, and DIM at diagnosis. Standing time increased on the day before mastitis diagnosis ($+1.2$ h/d; SE = 0.2; $P < 0.001$), returning to baseline on the day following diagnosis, and was greater overall when cows had pasture access ($+0.4$ h/d; SE = 0.3; $P < 0.001$). Step count decreased coinciding with the day of diagnosis, particularly in cows with pasture access ($d -1$ vs. $d 0$: 2,108 vs. 1,398 steps, SE = 128, with pasture access; 1,333 vs. 1,093 steps, SE = 82, with no pasture access; $P < 0.001$), returning to baseline at 7 d following diagnosis. Standing time decreased with concurrent lameness (-0.4 h/d; SE = 0.3; $P = 0.05$) or retained placenta (-1.9 h/d; SE = 0.4; $P < 0.001$), and step counts decreased with concurrent metritis (-363 steps/d; SE = 120; $P < 0.001$) or physical injury (-352 steps/d; SE = 123; $P = 0.02$). Standing bout frequency was not affected by mastitis or pasture access. In conclusion, these findings suggest that increased standing time and reduced step

count may predict mastitis, and that these behavioral changes may be amplified when cows have access to pasture.

Key Words: activity, mastitis, organic

2020 Prevalence and management of lameness on Iranian intensive dairy cattle farms. A. Jafari-Ghavamabad*¹, R. Laven¹, S. Jafari-Ghavamabad², F. Khaloubaghery³, S. M. H. Mirrahimi⁴, K. R. Mueller¹, M. Dehghan Banadaky⁵, and E. Vallee¹, ¹*School of Veterinary Science, Massey University, Palmerston North, New Zealand*, ²*Animal Science Department, College of Agriculture and Natural Resources, Karaj Islamic Azad University, Karaj, Iran*, ³*Independent Researcher, Palmerston North, New Zealand*, ⁴*School of Veterinary Medicine, Karaj Islamic Azad University, Karaj, Iran*, ⁵*Animal Science Department, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran*.

Lameness is one of the major welfare issues on dairy farms around the world, with an average prevalence of 22% across all farms. Intensive dairy cattle farming started around 80 years ago in Iran. Inspired by the US system, but there is still a lack of data available regarding the prevalence of lameness and its management. Thus, we aimed to develop and use a welfare assessment protocol, consisting of animal-based and resource-based measures as well as farm records, including those related to the management and prevalence of lameness. In 2022–2023, 62 farms were visited. Locomotion scoring was performed using a 4-point system at parlor exit. All cows with days in milk <150 were scored. Score ≥2 was considered lame. Descriptive analysis was done using SAS 9.4. As shown in Table 1, there was a notable difference between the actual and farm-recorded prevalence of lameness. Most farms (59%) hired a contractor hoof trimmer, and 41% had hoof trimming personnel. Of the farms that hired a contractor, 15% performed trimming >2 times/yr, but of those with an on-farm trimmer, 38% performed trimming >2 times/yr. Only one farm did not trim the hooves, with the farmer saying that he did not “believe in trimming.” This farm had 86% overall lameness and 21% severe lameness (the highest prevalence). Our data, as well as other studies, show that hoof trimming is a routine procedure on most Iranian farms, yet there is a lack of institutional hoof trimming education. In addition, 57% of farms had a foot bath and used it several times a week, and all farms reported using formaldehyde. This is comparable to dairy farms in Minnesota, where 68% of farms used foot baths and mostly used copper sulfate and zinc sulfate. Finally, our data suggest a high prevalence of lameness in early-lactation cows and a lack of reliability of farm-recorded data, which is similar to previous reports. Further investigation is needed to see whether the prevalence of lameness on Iranian farms is associated with trimming frequency on the farms.

Key Words: cattle welfare, hoof management, hoof trimming

2021 Rumen-derived microbial product supplementation: Effects on feed sorting in dairy cows. M. Bulnes-Larsen*¹, A. Celemin Sarmiento¹, T. H. Swartz¹, and M. E. Uddin², ¹*South Dakota State University, Brookings, SD*, ²*University of Connecticut, Storrs, CT*.

This study evaluated the effects of supplementing a rumen-derived microbial product (MP; *Clostridium beijerinckii* at 2×10^6 cfu/g, *Pichia*

kudriavzevii at 2×10^7 cfu/g, *Ruminococcus bovis* at 2×10^7 cfu/g, and *Butyrivibrio fibrisolvens* at 2×10^7 cfu/g) on feed sorting behavior in lactating dairy cows. Holstein cows were blocked by parity, DIM, and milk yield in a randomized complete block design with a 2-wk covariate period and 17 wk of treatment supplementation. Cows received either a control diet (CON, n = 17; TMR + 150 g/d ground corn) or the treatment (MP, n = 17; TMR + 150 g/d ground corn + 5 g/d MP). Samples of TMR and orts from a randomly selected subset of cows (CON, n = 10; MP, n = 8) were taken for 3 consecutive days in wk 16 and subjected to particle size analysis using a Penn State Particle Separator with 3 screens (19 mm, 8 mm, and 4 mm) and a bottom pan, which categorized particles as long, medium, short, and fine, respectively. Intakes were calculated as a percentage of covariate body weight. Sorting index was calculated as actual intake relative to predicted intake for each particle size fraction. Data were analyzed using the GLIMMIX procedure in SAS, including the fixed effect of treatment and the random effect of feeding order. Compared with CON, MP cows tended to consume more long particles ($P = 0.07$) but less short particles ($P = 0.06$) as a percentage of body weight. Treatment did not affect DM, NDF, physically effective NDF, medium, and physically effective particle (PEP) intake ($P > 0.22$). Cows supplemented MP sorted for long, medium, and PEP (103.1 vs. 97.5 ± 0.89 , $P < 0.001$; 100.3 vs. 98.8 ± 0.45 , $P = 0.03$; 100.8 vs. 99.54 ± 0.40 , $P = 0.03$) compared with CON. Conversely, MP cows sorted against fine particles (99.36 vs. 100.14 ± 0.25 , $P = 0.04$). Treatment did not affect feed sorting of short particles ($P = 0.57$). Supplementing a rumen-derived microbial product altered feed sorting behaviors, with a slight preference for consuming physically effective particles.

Key Words: direct-fed microbial, feed sorting, physically effective particles

2022 Are cows good moms? Exploring maternal behavior in multiparous versus primiparous cows. J. L. Byrd*, M. T. DeHaven, K. Turner, and H. W. Neave, *Purdue University, West Lafayette, IN*.

The objective of this study is to compare maternal behavior of multi- (n = 4) and primiparous (n = 4) Holstein dairy cows. Cow-calf pairs were housed in individual box stalls. Calves (birth weight range: 29.0–42.6 kg) were bottle-fed 4 L of colostrum (Brix score >22) within 8 h. Cows were milked twice daily. Video was recorded for 24 h after cow-calf pairs entered the box stall, and sampling was performed continuously for 4 behaviors: touching duration (muzzle of cow in contact with calf), allogrooming duration (cow licking calf), number of calf checks (cow reorientation toward calf followed by touching), and aggression occurrence (cow pushes or kicks calf). Touching, allogrooming and calf checks were analyzed with a linear regression model (parity and birth weight as fixed effects), and aggression occurrence (binomial variable) with a Fisher exact test. Multiparous (vs. primiparous) cows tended to touch their calves more (13.2 ± 1.9 min/24 h, range: 6.4–18.2 min/24 h; vs. 7.4 ± 2.1 min/24 h, range: 6.3–8.6 min/24 h; respectively; $P = 0.1$) with one extreme primiparous outlier removed from analysis (103.6 min/24 h). There was no difference in allogrooming between multi- and primiparous cows (65.2 min/24 h, range: 27.1–108.2 min/24 h; vs. 93.7 ± 13.8 min/h, range: 40.1–133 min/24 h; respectively; $P = 0.2$); however, lighter calves received more allogrooming ($P = 0.03$). Number of calf checks did not differ between multi- and primiparous

Table 1 (Abstr. 2020). Farm-recorded and actual prevalence of lameness (%) on Iranian intensive dairy farms

Lameness prevalence	1st quartile	Median	3rd quartile	Minimum	Maximum	N of farms
Farm recorded	6.1	8.3	18	1.7	39	32
Actual	26	33	42	10	86	61

cows (49 checks/24 h, range: 34–61 checks/24 h; vs. 64 ± 8 checks/24 h, range: 35–86 checks/24 h; respectively; $P = 0.2$); however, lighter (vs. heavier) calves tended to be checked more frequently ($P = 0.09$). There was no parity effect on aggression occurrence ($P = 0.5$). Overall, measures of maternal behavior did not differ between multi- and primiparous cows, but our sample size is limited, with large variation in maternal behavior expression. Some maternal behaviors were more directed at lighter vs. heavier calves, possibly to induce vigor, but this remains to be understood.

Key Words: welfare, early life, cow-calf

2023 Modeling effects of heat stress on dairy cattle lactation using local weather data and voluntary milking system records.

M. Malloy, M. E. Uddin, P. Rezamand, and B. Fragomeni*, *University of Connecticut, Storrs, CT.*

Heat stress in dairy cattle negatively affects milk production and reproductive efficiency. In turn, this reduces profitability and presents an increasingly major concern for the US dairy industry. The objective of this study was to analyze the effects of heat stress in dairy cattle on milk production using weather data at a dairy facility in the Northeastern United States. Data consisted of daily milking records from 100 Holstein cows from June 2020 to October 2022 from the Kellogg Dairy Center at the University of Connecticut (Storrs, CT). Weather data were collected from Windham Airport and from a local weather station installed at the farm. Using the weather data, the temperature-humidity index (THI) was computed for each day, and a heat load function was calculated when the THI exceeded the threshold of 65. Numerous linear models were run with milk yield as a function of multiple variables, including parity, lactation stage, season, year, and heat load. The results were validated using data collected one day of the week to predict milk yields for the remainder of the week. The correlation between THI calculated from weather data collected at Windham Airport and the Kellogg Dairy Center was 0.967 ($P < 0.05$). In all linear models, the heat load effect was significant ($P < 0.05$), and the model with the best fit included the effects of heat load nested in parity and stage in addition to year and season. The effects of heat stress were more severe during the first lactation and during the peak stages of lactation. Overall, the results indicate that milk yield is negatively affected by heat stress, and using data from a nearby weather station and voluntary milking system provides a framework for evaluating the effects of heat stress on production. Although airport weather data were used for this study for the sake of completeness, it is encouraged to collect weather data from a station located at the dairy facility. The results of this study serve as an example of how data from a voluntary milking system can be used to model heat stress in dairy cattle.

Key Words: temperature-humidity index, heat load, linear model

2024 Behavioral changes in dairy sheep with subclinical mastitis.

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Mastitis is an important disease in dairy sheep flocks, causing substantial economic losses and animal welfare concerns. In this study, we investigated whether subclinical occurrences of the disease affect ewes' activity levels and lying behavior. We used leg-mounted HOBO Pendant G Data Loggers to measure acceleration and tilt in 3 axes (x, y, and z) in 46 ewes with subclinical mastitis and 48 healthy, parity-matched control ewes, all between 3 to 28 d in milk. Subclinical mastitis was defined as absence of clinical signs but a positive California Mastitis Test (CMT) in at least 1 udder half or a trace result in both halves. Logger

data were collected at 5-s intervals over a 17-h period from 1000 h to 0300 h, excluding the afternoon milking from 1500 h. to 2000 h. These data were used to calculate the average lying time (min/h) and activity (vector sum of acceleration across all axes) for each ewe. We used linear models to evaluate the effect of subclinical mastitis on each behavior. Ewes with subclinical mastitis tended to spend more time lying (38.0 ± 1.40 min/h) than healthy ewes (34.6 ± 1.37 min/h; $F_{1,92} = 3.11$, $P = 0.08$), although overall activity did not differ between groups ($F_{1,92} = 1.467$, $P = 0.23$). Ewes with subclinical mastitis may lie more due to pain or fatigue, indicating a compromised affective state and overall welfare. The observed trend also highlights the potential for using accelerometers as a diagnostic tool for subclinical mastitis.

Key Words: accelerometer, lying, California Mastitis Test (CMT)

2025 Effect of separation methods on lamb behavior.

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The objective of this study is to evaluate the effects of different separation methods on lamb behavior. For this, 20 lambs, aged 27.75 ± 5.27 d, were divided into 3 groups. Control group ($n = 6$): lambs remained with their mother without any separation. Group 2 ($n = 7$): lambs stayed with their mother but were prevented from suckling using a nasal ring. Group 3 ($n = 7$): lambs were separated from their mother by a physical barrier. The experiment lasted 3 d, with the separation period set at 12 h/d (from 2000 h to 0800 h). Two lambs from each group were equipped with an accelerometer (device already validated for the study of sheep behavior). An algorithm was developed to detect 4 types of behaviors: lying down, standing still, walking, and playing. Lambs separated from their mother (groups 2 and 3) showed more signs of stress and spent less time lying down, with a more pronounced response when they were separated without physical contact with their mother (group 3). Group 1 spent more time ($P < 0.05$) playing and lying down than groups 2 and 3. Group 3 spent 50% of its time walking and remained standing 4 times longer than group 1 and twice as long as group 2. These results indicate that lamb behavior varies depending on the separation method. This study provides practical recommendations for improving animal welfare in livestock management, suggesting that separation with a nasal ring (group 2) may be a more suitable alternative to complete physical separation.

Key Words: lamb, behavior, stress at separation

2026 Spray of eucalyptus and mint essential oils on Jersey cows during transition period: Effects on animal well-being and early-lactation production.

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Heat stress is a challenge to lactating dairy cows during summer in subtropical regions, affecting cow well-being, milk production, and efficiency. The effects of spraying a combination of eucalyptus and mint essential oils (EO) during the pre- and postpartum periods on animal well-being and production efficiency were evaluated. Fourteen second-pregnancy Jersey cows (410 kg live weight) were housed in a prepartum barn until calving. The experimental period lasted 60 d, comprising 30 d before the expected calving date and the first month

of lactation. After calving, the cows were moved to a compost barn. Cows were assigned to 2 treatments: control (water only) and essential oil (EO: water plus eucalyptus and mint EO) in a completely randomized design. Data analysis was conducted using SAS with the MIXED procedure and Satterthwaite approximation. Spraying (15 min daily) was performed daily between 1330 h and 1430 h, in a 12-m² cooling room with a 2 HP pump system and 9 spray nozzles (2.4 L/min). Control cows were sprayed first, followed by EO cows after a 20-min interval, to prevent cross-contamination of the EO spray. Cows were milked twice daily (VMSTM V300, DeLaval). Milk production and DMI were measured daily. Milk samples were collected at 1, 5, 10, 15, 20, 25, and 30 DIM to analyze SCC and milk solids. Respiratory rate (RR), rectal temperature (RT), and skin surface temperature (rump, flank, and leg) were measured weekly over 2 consecutive days before spraying, 15 min (T-15), and 60 min (T-60) after spraying. The EO treatment reduced skin surface temperatures at T-15 ($P < 0.05$) and RR at both T-15 and T-60 ($P < 0.05$), with no effect on RT ($P = 0.69$). Compared with the control group, EO-treated cows showed no significant differences ($P > 0.05$) in daily milk production (24.0 vs. 21.4 kg), daily DMI (11.4 vs. 11.1 kg), milk solids, or SCC (48.6 vs. 58.5×10^3 cells/mL). However, EO cows produced more milk during the first 30 DIM (721 vs. 644 kg/cow; $P = 0.03$) and showed a trend toward improved feed efficiency (2.11 vs. 1.93; $P = 0.06$). The EO mix showed promising potential for improving cow well-being and early-lactation performance.

Key Words: essential oil, feed efficiency, skin temperature

2027 The first 24 hours: Nursing and suckling behaviors of cows and calves, and possible need for human assistance. M. T. DeHaven*, J. L. Byrd, K. Turner, and H. W. Neave, *Purdue University, West Lafayette, IN*.

This study assessed early nursing behavior of primi- and multiparous dairy cows, suckling behavior of their calves, and the possible need for human assistance to suckle. Cow-calf pairs ($n = 4$ each, primi- and multiparous) were housed in individual box stalls upon birth of the calf. Calves (BW range: 29.0–42.6 kg) were bottle-fed colostrum (4 L; ≥ 22 Brix) after successful suckling (assisted or independent) within 8 h of birth, and were assisted to suckle before milking (twice daily) if they did not suckle independently. Human assistance involved positioning the calf in inverse parallel and leading to the teat until independent suckling or diminished suckle reflex. Four behaviors were recorded and continuously analyzed over 24 h: cow nursing avoidance (kick or move away from calf), successful suckling duration, teat-seeking attempts, and human assist to suckle duration. Linear regression models analyzed behavioral differences between primi- and multiparous cows (fixed effects: parity, calf BW). Parity did not affect nursing avoidance (primiparous: 16.9 ± 3.4 events/24 h [range: 14–26] vs. multiparous: 8.6 ± 3.4 events/24 h [range: 4–17]; $P = 0.15$), nor suckling duration (primiparous: 21.0 ± 7.8 min/24 h [9.8–33.4] vs. multiparous: 17.0 ± 7.8 min/24 h [0.37–36.1]; $P = 0.73$). Parity also did not affect teat-seeking attempts (primiparous: 29.0 ± 4.4 attempts/24 h [17–42] vs. multiparous: 29.7 ± 4.4 attempts/24 h [18–48]; $P = 0.92$), nor human assist to suckle duration (primiparous: 11.2 ± 2.9 min/24 h [0–22.7] vs. multiparous: 5.6 ± 3.3 [0–16.2]; $P = 0.28$). One extreme outlier (multiparous: 47.2 min/24 h) was excluded from analysis of human assist to suckle. Lighter (vs. heavier) calves tended to have fewer teat-seeking attempts ($P = 0.06$) and required more human assistance to suckle ($P = 0.04$). Overall, primi- and multiparous cows did not differ in nursing avoidance behavior, and their calves had similar teat-seeking attempts and suckling durations. However, lighter calves may require more time to teat-seek and may need more assistance from human caretakers if implementing extended cow-calf contact.

Key Words: cow-calf, postpartum, welfare

Animal Health 1: Posters

2028 Comparison of cow-calf contact or transition milk feeding on calf growth and health scores. R. Ewing^{*1}, A. Beard¹, G. Frederick¹, and Á. Abuelo², ¹Cornell University College of Veterinary Medicine, Ithaca, NY, ²Michigan State University, East Lansing, MI.

Our objective was to evaluate the effects of 3 early-life calf rearing methods in a randomized block design: (1) unrestricted cow-calf contact (URS, $n = 18$), or separation at birth but fed (2) dam's transition milk (TM, $n = 16$) or (3) bulk tank whole milk (WM, $n = 20$) for the first 5 d. Singleton Holstein or beef cross calves ($n = 54$) were fed their dam's colostrum (mean Brix [$\pm$ SE] of $27.2 \pm 0.5\%$) at 8% of birth bodyweight (BW) <2 h of parturition. Pairs were housed individually, with URS having full dam access, whereas TM and WM pairs were separated, housed identically, and offered TM or WM at an amount equivalent to 10% of BW 2 $\times$ /d. On d 5, URS calves were separated and all calves moved to outside hutches and offered WM at 10% of BW 2 $\times$ /d. In the first week, health scores (0–3, higher is worse), fecal scores (0–3, higher is worse), rectal temperature, milk intake, and BW were recorded daily, whereas withers and hip heights were taken at d 0 and d 7. From wk 2–9, BW, frame, and health scores were recorded 1 $\times$ /wk in Holstein females only ($n = 28$: URS = 8, TM = 9, WM = 11). Statistical analysis was conducted as a mixed effects ANOVA, with repeated measures as appropriate, with fixed effects of treatment (trt), day, and trt $\times$ day, and random effect of block (JMP Pro 17). Fecal scores were analyzed separately from other health scores. Data are reported as LSM $\pm$ SE. On d 1–4, milk consumption was similar for TM and WM on a kilograms-consumed basis ($P = 0.69$; TM: 7.5 ± 0.38 kg, and WM: 7.1 ± 0.34 kg on d 4) and percent BW basis ($P = 0.62$; TM: $18.2 \pm 1\%$, and WM: $17.5 \pm 1\%$ on d 4). There was no effect of trt on health scores from d 0–7 ($P = 0.18$) or fecal scores from d 1–7 ($P = 0.89$), but fecal scores were higher on d 2 than d 4–7 ($P < 0.02$). From wk 2 to 9, there was no effect of trt on weekly health or fecal scores ($P \geq 0.27$). The average daily gain (ADG) at 9 wk of age was 0.83 ± 0.03 kg/d (URS), 0.81 ± 0.03 kg/d (TM), and 0.83 ± 0.03 kg/d (WM), and was not affected by trt ($P = 0.84$). Frame growth was not affected by treatment ($P \geq 0.35$). Rearing calves with cow-calf contact or TM feeding until d 5 did not affect calf health and growth compared with standard WM practice.

Key Words: transition milk, cow-calf contact, early life

2029 Machine learning-driven thresholds to identify newborn calves with enlarged umbilical stumps. A. Rico^{*1,2}, A. F. A. Pires¹, and N. Silva-del-Rio^{1,2}, ¹Department of Population Health and Reproduction, UC Davis School of Veterinary Medicine, Davis, CA, ²UC Davis Veterinary Medicine Teaching and Research Center, UC Davis School of Veterinary Medicine, Davis, CA.

Omphalitis prevalence in dairy farms is underdiagnosed. Research settings have used umbilical stump diameter as a diagnostic criterion, but threshold selection has been arbitrary. Machine learning, via unsupervised clustering, offers a novel approach to characterizing enlarged and non-enlarged subpopulations. This study aims (1) to assess the intraobserver and interobserver reliability of a technique for measuring umbilical stump diameter and (2) to propose data-driven thresholds for defining enlarged navels in Holstein (HO) and Jersey (JE) heifers aged 3–10 d. The umbilical stump diameter was measured using a caliper positioned latero-laterally as close as possible to the external umbilical ring. For intraobserver reliability, 157 4-d-old female calves were measured 3 times by one rater. For interobserver reliability, 44 calves

were measured once by 3 raters. To establish thresholds, we enrolled 667 female calves (HO: 437; JE: 230) from 17 dairies. Statistical analysis was performed in R. Intraobserver agreement was assessed using the coefficient of variability (CV), and interobserver agreement using the intraclass correlation coefficient (ICC). Thresholds were estimated separately for each breed using finite mixture models, expectation maximization, parametric simulations, and receiver operating characteristic (ROC) analysis, assuming 2 lognormal distributions representing nonpathological and pathological populations. The intraobserver agreement was classified as very good (CV = 5.94%, 95% CI: 5.31–6.57), and the interobserver agreement was fair to good (ICC = 0.76, 95% CI: 0.65–0.85). The ROC analysis showed areas under the curve of 0.62 (95% CI: 0.56–0.67) for HO and 0.74 (95% CI: 0.67–0.81) for JE. The optimal thresholds, balancing sensitivity (Sn) and specificity (Sp), were 16.6 mm for HO and 12.6 mm for JE, with Sp $\sim$ 0.95 but fair to poor Sn (0.38 in HO, 0.61 in JE). A positive predictive value (PPV) of 0.99 could be achieved by increasing thresholds to 19.7 mm for HO and 14.6 mm for JE. Measuring umbilical stump diameter at the external umbilical ring is precise and shows potential discriminatory power for identifying enlarged navels. The most adequate approach is to set thresholds based on predictive accuracy. The proposed thresholds, particularly those achieving a PPV of 0.99, should be applied at the herd level to improve calf health through population-oriented decisions.

Key Words: omphalitis, umbilical disease, machine learning

2030 Evaluating a rapid immunity test to predict dairy calf mortality risk. A. M. Roper^{*1}, C. Guzi Savegnago¹, T. Nogueira Marins¹, J. Gao¹, R. Xie², S. Tao¹, and Q. Huo³, ¹Department of Animal and Dairy Science, University of Georgia, Athens, GA, ²Department of Statistics and Data Science, University of Central Florida, Orlando, FL, ³Department of Chemistry and NanoScience Technology Center, University of Central Florida, Orlando, FL.

Transfer of passive immunity (TPI) is critical for the survival of preweaning dairy calves. Plasma or serum Brix value measured by a refractometer is widely used to estimate circulating immunoglobulin (Ig) G concentration as a proxy for TPI. However, the Brix value estimates only the total solid percentage in the circulation and cannot represent the immune status of the calf. The D2Dx immunity test is a nanotechnology-based immune response test to measure humoral immunity of animals. The objective of this study was to compare the ability of Brix values or D2Dx test scores to predict mortality risk of calves within 30 d of age (DOA). Over a 15-mo period, heifer calves at 2 DOA were enrolled from a farm with low calf mortality rate ($n = 849$, 2.9% mortality) and a farm with high calf mortality rate ($n = 698$, 11.7% mortality). At enrollment, serum was collected to perform D2Dx immunity testing and measure Brix values. The D2Dx score and Brix values of serum samples were compared between calves that died from 3 to 30 DOA and calves that survived, using the GLM procedure of SAS. In both low- and high-mortality farms, calves that survived within 30 DOA had greater serum Brix values (9.05 vs. 8.48, and 9.26 vs. 8.99) and D2Dx scores (0.0281 vs. 0.0036, and 0.0342 vs. 0.0286) on 2 DOA compared with calves that died. The D2Dx scores were significantly associated with Brix values regardless of mortality in both farms (Spearman's $\rho = 0.42$, $P < 0.001$). Further, we used logistic regression with R to predict the mortality outcome (dead vs. alive), using D2Dx scores and Brix values as the predictor. Data from the low-mortality farm suggested that

the D2Dx score has better sensitivity (96 vs. 48%) and area under the curve (0.87 vs. 0.69) than the Brix value in predicting calf mortality. However, similar results were not observed in data collected from the high-mortality farm, possibly because there were other issues in addition to TPI that influenced calf survival in the high-mortality farm. We conclude that the D2Dx immunity test may be used as a predictor for calf mortality risk. However, the prediction is most effective in a farm with low mortality.

Key Words: D2Dx immunity test, passive immunity, mortality

2031 Impact of heat stress abatement on cytokine production by immune cells and in the bronchoalveolar lavage fluid following bovine respiratory disease in preweaning dairy calves. C. G. Savegnago^{*1}, A. M. Roper¹, T. N. Marins¹, J. Gao¹, G. G. Cunha¹, N. L. P. Kant¹, C. R. Czaykowski², B. Credille², M. M. Woldemeske³, and S. Tao¹, ¹Department of Animal and Dairy Science, University of Georgia, Athens, GA, ²College of Veterinary Medicine, University of Georgia, Athens, GA, ³Veterinary Diagnostic and Investigational Laboratory, University of Georgia, Tifton, GA.

Heat stress negatively affects immune function and may increase mortality risk of bovine respiratory disease (BRD). We aimed to examine the impacts of heat abatement during summer on cytokine production by immune cells *ex vivo* and in the bronchoalveolar lavage fluid (BALF) following experimentally induced bacterial bronchopneumonia in preweaning calves. Holstein bull calves ($n = 16$, days of age = 21) were assigned to 2 treatments based on body weight and serum Brix refractometry: (1) non-cooled (NC, $n = 8$); (2) cooled (CL, $n = 8$). The CL calves were housed under a barn and cooled with fans, and NC calves were housed adjacent to the barn without shade or fans. Milk replacer was offered twice a day and calf starter provided *ad libitum*. Blood was drawn at 3 and 17 d of the experiment (DOE) to isolate peripheral blood mononuclear cells (PBMC). Cells were stimulated by concanavalin A (ConA) and lipopolysaccharide (LPS) for 48 h to collect supernatant for cytokine analysis. On 22 DOE, all calves were inoculated with 10^7 cfu *Mannheimia haemolytica* at the tracheal bifurcation. Calf BALF was collected at 0, 6, 12, 24, 72, and 168 h following inoculation to analyze cytokines. Calves were sacrificed at 240 h, and visual lung lesions were scored. Data were analyzed using linear mixed-effects models of SAS. No treatment effects ($P = 0.72$) in cytokine production of PBMC stimulated by LPS were observed. Relative to CL, NC PBMC stimulated by ConA tended ($P = 0.06$) to produce more TNF α (1.2 vs. 1.7 ± 0.3 ng/mL) and IL1 β (0.18 vs. 0.28 ± 0.03 ng/mL) at 3 DOE. The IL1 α concentration in CL BALF was greater (48.4 vs. 13.4 ± 15.6 pg/mL; $P = 0.01$) at 6 h but lower (18.8 vs. 173.2 ± 68.3 pg/mL; $P = 0.01$) at 168 h following BRD than that of NC. At 168 h after BRD, CL BALF tended (3.2 vs. 48.0 ± 27.1 ng/mL; $P = 0.08$) to have lower TNF α concentration than NC. At euthanasia, CL calves had lower ($P = 0.02$) lung lesion scores than NC. In conclusion, heat abatement may promote inflammation resolution in the lung following BRD, reducing disease severity.

Key Words: bovine respiratory disease (BRD), dairy calf, heat abatement

2032 Effects of weaning pace and probiotic supplementation on mRNA abundance of inflammation-related genes in rumen and liver tissue of male Holstein dairy calves. M. Malekhhahi^{*1}, H. Rasmussen¹, D. Konetchy¹, M. Mahdavi-Yekta¹, M. M. Larson¹, A. Ahmadzadeh¹, A. H. Laarman^{1,2}, L. Guan³, and P. Rezamand^{1,4}, ¹University of Idaho, Moscow, ID, ²University of Alberta, Edmonton,

AB, Canada, ³University of British Columbia, Vancouver, BC, Canada, ⁴University of Connecticut, Storrs, CT.

The weaning transition imposes stressors on dairy calves affecting immune function. Probiotic supplementation has been proposed as a method to improve the immune system. The objective was to investigate the effects of weaning pace and probiotic supplementation (*Ligilactobacillus agilis*, *Lactobacillus delbrueckii*, *Limosilactobacillus mucosae*, and *Limosilactobacillus reuteri* at 1×10^9 cfu/d) on the mRNA abundance of inflammation-related genes in rumen and liver tissues of male Holstein calves. Thirty-eight calves were blocked by birth weight and assigned randomly to a 2×2 factorial arrangement: (1) abrupt weaning without probiotics (AC; $n = 10$), (2) abrupt weaning with probiotics (AP; $n = 10$), (3) gradual weaning without probiotics (GC; $n = 9$), and (4) gradual weaning with probiotics (GP; $n = 9$). Abrupt weaning was performed over 3 d (54–57 d) with 3 step-downs of milk replacer at 1.13 L per feeding. Gradual weaning occurred over 14 d (49–63 d) with 7 step-downs of milk replacer at 0.49 L per feeding. Probiotics were supplemented in the milk replacer (22% CP, 22% fat) for 7 d, starting 4 d before weaning. Calves had *ad libitum* access to water and were fed calf starter (18% CP) and chopped alfalfa hay. Calves were euthanized 1 day after weaning, and rumen and liver tissue samples were taken to determine the mRNA abundance of inflammation-related genes: interleukin 1 β (*IL-1 β*), interleukin 6 (*IL-6*), interleukin 8 (*IL-8*), tumor necrosis factor- α (*TNF- α*), interferon-gamma (*IFN- γ*), nuclear factor kappa B (*NF- κ B*), and intercellular adhesion molecule 1 (*ICAM1*). Data were analyzed by 2-way ANOVA and pairwise comparisons ($P < 0.05$). No differences were found in gene expression in the rumen between the weaning methods. However, AP calves showed an increased mRNA abundance of *IL-6* in the rumen ($P = 0.01$) and tended to have a greater mRNA abundance of *IFN- γ* ($P = 0.06$) and *ICAM1* ($P = 0.09$) compared with AC calves. In addition, abrupt weaning showed reduced mRNA abundance of *IL-6* ($P = 0.065$) and *NF- κ B* in the liver ($P = 0.1$). Overall, the decreased mRNA abundance of *IL-6* in the liver in abruptly weaned calves indicates decreased systemic inflammatory response. Probiotic supplementation, however, increased ruminal inflammation-related gene expression, indicating a localized immune response.

Key Words: weaning pace, probiotic, gene expression

2033 Offering 750 g of milk replacer at 16.5% or 12.5% dry matter does not affect the intake and performance of Holstein calves. J. Diavão^{*1}, S. M. Mendes², M. M. Campos¹, L. A. J. Silva³, S. E. Ferreira³, A. S. Silva¹, H. F. Lage², and S. G. Coelho², ¹Empresa Brasileira de Pesquisa Agropecuária-Embrapa Gado de Leite, Juiz de Fora, MG, Brazil, ²Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil, ³Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brazil.

The objective of this study was to evaluate the effect of density (ADS) and dilution of the liquid diet (VOL) with the same amount of nutrients on the intake, digestibility, and performance of Holstein calves. Thirty calves (18 males and 12 females) were housed in individual pens and randomly assigned to 1 of 2 treatments: (a) VOL, 6 L/d, 12.5% DM (750 g Nurture Prime, 21.4% CP and 14.8 ether extract [EE]); and (b) ADS, 4.5 L/d, 16.7% DM (750 g Nurture Prime). Daily milk replacer intake and daily intakes of drinking water and starter (95.18% DM, 20.65% CP, 3.20% EE) were evaluated until 60 d. Two digestibility trials were performed (30 to 34 and 52 to 56 d of age) in individual metabolic cages (1.50 $\times$ 0.80 m, Ponta Ltda. Contagem, Brazil) from d 30 to 34 and 52 to 56. Total feces collection was performed to estimate the DM, OM, CP, EE, and NDF digestibility. Average daily gain (ADG; ICS 300, Coinma, Dracena, Brazil) was evaluated weekly. The data were

analyzed using the R statistical package (R Core Team, 2019) and the means were compared using the Tukey test with significance $P \leq 0.05$, with sex and birth weight used as covariables. The VOL treatment showed higher digestibility of GE (VOL = 97.4% vs. ADS = 96.5%, $P < 0.05$) in the first digestibility trial. Both ADG (VOL = 662.4 g vs. ADS = 650.1 g) and intake parameters were similar between treatments. Given the results observed, the producer can choose when offering 750 g of DM of replacer between thickening 16.5% DM or diluting the diet 12.5% DM without harming calf performance.

Key Words: calf raising, weaning

2034 Effects of calf gut-originated probiotics and weaning pace on subjective health measures, serum non-esterified fatty acids, and plasma haptoglobin in Holstein dairy calves. H. Rasmussen¹, D. Konetchy¹, M. Mahdavi-Yekta¹, M. Larson¹, J. Ranson^{*1}, A. Ahmadzadeh¹, A. H. Laarman^{1,2}, L. Guan³, and P. Rezamand^{1,4}, ¹University of Idaho, Moscow, ID, ²University of Alberta, Edmonton, AB, Canada, ³University of British Columbia, Vancouver, BC, Canada, ⁴University of Connecticut, Storrs, CT.

Weaning is a stressful event for a calf and can lead to increased morbidity. With increasing concerns about antimicrobial usage and resistance, alternative methods of disease treatment and prevention are needed. This study examined the effects of probiotic supplementation using a calf gut-originated direct-fed probiotic treatment (*Lactobacillus agilis*, *Lactobacillus delbrueckii*, *Lactobacillus mucosae*, and *Lactobacillus reuteri*, 1×10^9 cfu/d; control vs. supplemented) and varied weaning pace (abrupt vs. gradual) in a 2×2 factorial arrangement of treatments on subjective health and hematological measures in dairy calves. Holstein bull calves ($n = 38$) were blocked by birth weight and assigned randomly to 1 of 4 treatments: abrupt-control (AC; $n = 10$), abrupt-supplemented (AP; $n = 10$), gradual-control (GC; $n = 9$), and gradual-supplemented (GP; $n = 9$). Calves were fed a total of 3.4 L of milk replacer (22% CP, 22% fat) twice daily. The probiotic was supplemented in milk replacer once daily for 7 d, beginning 4 d preweaning. Abrupt weaning occurred over d 54–57 of age in 3 incremental step-downs of 1.13 L. Gradual weaning occurred over d 49–63 of age in 7 incremental step-downs of 0.49 L. Subjective health observations were scored using a 1–4 scale (1 = healthy, and 4 = debilitated) including cough severity, facial discharge, depression of ears, fecal consistency, joint and navel inflammation, and attitude measured daily; hydration status and perineal cleanliness were measured weekly. Serum nonesterified fatty acids (NEFA) and plasma haptoglobin were measured at d 3 and 7 of age, onset of treatment, and 1 d postweaning. Data were analyzed using a mixed model in SAS, with pace and supplementation as fixed effects. Results showed no detectable differences in plasma haptoglobin concentrations, with values ranging from 0.161 to 3.297 mg/mL. Differences in NEFA concentrations and subjective health scores were not detected. Overall, there were no detectable effects observed in hematological or other health measures by probiotic supplementation or weaning pace under our experimental conditions.

Key Words: probiotics, weaning pace, antimicrobial resistance

2035 Optimizing serum IgG and total protein cut points by age for predicting morbidity and mortality in Holstein calves. H. M. Goetz^{*1}, M. A. Steele², H. R. McCarthy^{1,2}, and D. L. Renaud¹, ¹Department of Population Medicine, University of Guelph, Guelph, ON, Canada, ²Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.

The objective of this diagnostic accuracy study was to determine day-specific IgG and serum total protein (STP) cut points for predicting bovine respiratory disease (BRD), diarrhea, and survival in Holstein dairy calves ($n = 199$) fed colostrum replacer in Ontario, Canada. Blood samples were collected daily from d 1–7 of life and analyzed for serum IgG and STP. Calves were assessed for BRD and diarrhea daily until d 49, and mortality was recorded. Due to the high disease levels, calves were classified as having low ($\leq 18\%$ of days with BRD; $n = 111$) or high ($\geq 19\%$; $n = 83$) BRD prevalence and low ($\leq 12\%$ of days with diarrhea, $n = 108$) or high ($\geq 20\%$, $n = 89$) diarrhea prevalence, based on median disease duration. Youden's index was used to determine optimal cut points maximizing sensitivity (Sn) and specificity (Sp) for predicting low BRD and diarrhea prevalence and survival for each day between d 1–7 of life. The optimal IgG cut point for predicting survival decreased from 24.55 g/L (Sn = 0.64; Sp = 0.55; area under the curve [AUC] = 0.59) on d 1 to 14.05 g/L (Sn = 0.50; Sp = 0.52; AUC = 0.51) on d 7. Similarly, optimal STP cut points for survival declined from 5.55 g/dL (Sn = 0.59; Sp = 0.55; AUC = 0.57) on d 1 to 4.55 g/dL (Sn = 0.71; Sp = 0.38; AUC = 0.54) on d 7. For low BRD prevalence, the optimal IgG cut point declined from 24.35 g/L (Sn = 0.69; Sp = 0.40; AUC = 0.55) on d 1 to 11.85 g/L (Sn = 0.69; Sp = 0.43; AUC = 0.56) on d 7. A similar trend was found for STP, decreasing from 5.65 g/dL (Sn = 0.54; Sp = 0.49; AUC = 0.51) on d 1 to 4.65 g/dL (Sn = 0.67; Sp = 0.40; AUC = 0.54) on d 7. For low diarrhea prevalence, the optimal IgG cut point was 24.85 g/L on d 1 (Sn = 0.66; Sp = 0.47; AUC = 0.57) and 14.25 g/L on d 7 (Sn = 0.56; Sp = 0.70; AUC = 0.63). Similarly, the STP cut point for predicting low diarrhea declined from 5.65 g/dL on d 1 (Sn = 0.53; Sp = 0.48; AUC = 0.51) to 4.95 g/dL on d 7 (Sn = 0.46; Sp = 0.70; AUC = 0.58). These results highlight the importance of day-specific IgG and STP thresholds when predicting morbidity and survival in neonatal calves, emphasizing a need for age-adjusted reference values.

Key Words: passive immunity, colostrum, health prediction

2036 Factors impacting electrolyte usage, morbidity, and mortality rates in non-replacement dairy calves at a commercial calf rearing facility. H. McCarthy^{*}, M. A. Steele, and D. L. Renaud, University of Guelph, Guelph, ON, Canada.

This study aimed to explore the effects of breed, sex, arrival BW, and source on electrolyte usage, bovine respiratory disease (BRD), and mortality during the first 11 wk at a commercial calf rearing facility in southern Ontario (ON), Canada. The records of 6,879 calves (5,807 Angus $\times$ Holstein cross, 735 Holsteins, and 337 other breeds; 4,241 steers, and 2,638 heifers) were analyzed. Upon arrival, calves received 2.8 L of milk twice daily from d 1 until weaning (d 63) and had ad libitum access to water and grain. Orts were recorded after each feeding, and electrolytes were given to all calves with orts; antimicrobial treatment was administered to calves diagnosed with BRD during daily evaluations. Source was categorized based on calves picked up directly from dairy farms (routes: southern, central, and eastern ON, Quebec, and Maritimes), and auctions (southern ON and Quebec). Mixed logistic regression models were used to analyze the data, with week of arrival as a covariate. Compared with the southern ON route, the odds of BRD were higher for calves from southern ON auctions (odds ratio [OR] = 1.4; 95% CI: 1.1–1.7) and central ON routes (1.40; 1.1–1.8), whereas Quebec routes had the lowest odds of BRD (0.90; 0.65–1.25). Conversely, calves from Maritimes routes (1.4; 1.2–1.6) and Quebec auctions (1.2; 1.0–1.3) had increased odds of receiving electrolytes compared with southern ON-route calves. Arrival BW affected electrolyte usage, BRD, and mortality, where for every 1-kg decrease in BW, the odds of BRD, electrolytes, and mortality increased by 1.5% (0.985; 0.98–0.99),

2.4% (0.976; 0.97–0.98), and 2.7% (0.973; 0.94–0.98), respectively. Holsteins had higher odds of incurring BRD (2.1; 1.80–2.46) and mortality (2.0; 1.17–3.42) compared with Angus × Holsteins, and heifers had lower odds of BRD (0.79; 0.71–0.87) compared with steers. These data suggest that heavier Angus × Holstein heifers have the lowest odds of BRD, mortality, and electrolyte usage. Additionally, although electrolyte usage may be related to transport distance, BRD morbidity seems to be driven by other factors related to source.

Key Words: surplus calf, dairy-beef, morbidity

2037 Dairy calves from a lower-morbidity farm exhibit a more activated lung immune transcriptome in the preweaning period.

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Preweaning bovine respiratory disease (BRD) is one of the leading causes of sickness and death in dairy calves. Farm management dynamics strongly influence respiratory disease risk; however, the mechanisms of respiratory immune regulation in different farm environments have not been fully explored. Therefore, the objective of this study was to evaluate lung immune function in healthy calves from farms with differing morbidity rates. Preweaning calves were selected for bronchoalveolar lavage fluid (BALF) sampling based upon an absence of clinical respiratory disease signs, rectal temperature <39.4°C, and no lung consolidation on lung ultrasound. Calves were sampled from 2 farms: Farm A (HighM, n = 9, >20% preweaning BRD morbidity) and Farm B (LowM, n = 10, 10% preweaning BRD morbidity). All BALF samples were washed and filtered (70-µm filter), and cells were isolated for RNA extraction by centrifugation. Next-generation bulk Poly(A) transcriptomic sequencing (RNA-seq) was performed on all samples using a Novaseq 6000 sequencer (Illumina). Sequences were aligned to the bovine genome, ARS-UCD1.3, using a STAR alignment pipeline, and differential gene expression analysis was conducted in R using DESeq2. The HighM calves had 2.2 greater odds of developing clinical respiratory disease signs in the preweaning period than LowM ($P = 0.06$) and 6.2 greater odds of developing lesions on lung ultrasound ($P < 0.01$). Differential gene expression analysis demonstrated 277 upregulated genes in LowM calves, and 173 downregulated genes (false discovery rate <0.05; fold change >2). Gene Set Enrichment Analysis was performed using Gene Ontology: Biological Process gene sets to determine differentially expressed pathways. The LowM calves demonstrated upregulated pathways in leukocyte migration, T cell activation, and cellular adhesion, with key upregulated genes including *LCK*, *VCAMI*, and *CCL5*. In this study, healthy calves from a lower-morbidity farm exhibited a more activated lung immune transcriptome than healthy calves from a high-morbidity farm, indicating that immune activation may have played a protective role in preserving calf respiratory health.

Key Words: RNA sequencing (RNA-seq), immunology

2038 Dexamethasone-induced differentially expressed genes and pathways in leukocytes from heat-stressed Holstein calves.

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Heat stress phenotype is mediated by inflammation, and dexamethasone (dexa) is a synthetic glucocorticoid that reduces inflammation in dairy cattle. A study was conducted with 24 Holstein bull calves of

3.5 ± 0.5 weeks age and 68.5 ± 1.37 kg BW, to assess dexa-induced immune-related differentially expressed genes (DEGs) and pathways in heat-stressed (HS) dairy calves. Calves were individually housed in climate-controlled rooms with an ambient temperature (AT) of 19.8 ± 0.8°C for a 5-d acclimatization period. Based on their BW, ADG, and DMI, calves were randomly assigned to 1 of the 3 treatments (n = 8) for 5 d as (1) thermoneutral (TN; AT of 20°C throughout the day), (2) HS (AT of 40°C for 12 h followed by 27°C), and (3) HS dexa (HSD; AT of 40°C for 12 h followed by 27°C). The HSD group received dexa at a dose of 0.05 mg/kg BW, and TN and HS groups received saline at a dose of 1.2 mL on d 1 and 3 at 0700 h. Cyclic heat stress was imposed on HS and HSD groups from 0800 to 1900 h. Blood samples were collected on 0, 12, and 108 h of the study for RNA extraction, sequencing (n = 8; Takara SMART-Seq mRNA 3'DE using Illumina NovaSeq 6000 platform and ARS-UCD2.0 RefSeq) and transcriptomics analysis. The DEGs were called using a linear mixed effects model using R package with combinations of treatment and time point as a fixed effect, and animal as random effect with significance at false discovery rate-adjusted $P < 0.05$. The comparison between HS vs. TN treatments showed a total of 2 DEGs. A total of 980 DEGs were identified in HS compared with HSD. The pairwise comparison of HS vs. HSD at 12 h identified 1,789 upregulated and 920 downregulated DEGs. Notably, 66 immune-related genes were upregulated in HSD animals compared with HS animals, among which interleukins (IL-1RA, IL16, IL4R, IL1R2), TLR, and cluster of differentiation (CD) genes were predominant. In summary, the HSD treatment induced upregulation of genes associated with leukocyte activation phagocytosis, and immune system signaling, which may ameliorate the negative effects of heat stress in dairy calves' health and productivity.

Key Words: heat stress, dexamethasone, immune pathways

2039 Factors associated with diarrhea recovery in non-replacement dairy calves.

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The objective of this cohort study was to evaluate factors associated with recovery from diarrhea and antimicrobial use in non-replacement dairy calves at a calf-rearing facility. Upon arrival (3–10 d of age) to the facility, blood samples were collected to measure serum total protein (STP), and body weights were recorded. Calves were scored twice daily for fecal consistency and were enrolled at diarrhea onset, with fecal samples collected and tested via PCR for coronavirus, rotavirus, and *Cryptosporidium parvum*, and cultured for *Salmonella*. Further, all antimicrobial treatments (categorized for diarrhea or respiratory disease), and mortalities were recorded. Cox proportional hazards models assessed time to diarrhea resolution (2 consecutive fecal consistency scores of 0 or 1) and antimicrobial treatments. Among the 116 enrolled calves, 61.2% developed diarrhea during the first week, and 38.8% developed it in the second week. The mean (±SD) STP was 5.36 ± 0.72 g/dL. Pathogen prevalence was 37.4% for coronavirus, 66.1% for *C. parvum*, 87.0% for rotavirus, and 6.0% for *Salmonella*. Diarrhea resolved in an average of 4.49 ± 2.88 d, with timing of onset as the only significant predictor, where calves that developed diarrhea in the second week recovered faster (hazard ratio [HR]: 3.24; 95% CI: 2.07–5.08; $P < 0.001$) compared with those that developed diarrhea in the first week. Overall, 63.8% of calves were treated with an antimicrobial for diarrhea, with calves with STP >6.2 g/dL at arrival having a lower hazard of treatment (HR: 0.34; 95% CI: 0.13–0.89; $P = 0.03$) compared with those with STP <5.1 g/dL. Additionally, 36.7% were treated for respiratory disease after the diarrhea bout, with those developing diarrhea in the second week having a lower hazard of treatment (HR: 0.47; 95% CI: 0.23–0.97; $P = 0.04$).

compared with those with earlier diarrhea onset. No pathogens were associated with diarrhea resolution or antimicrobial use. These findings suggest that early-life factors, particularly timing of diarrhea onset and STP levels at arrival, may be more important in influencing diarrhea resolution and antimicrobial use than the pathogen type.

Key Words: diarrhea, antimicrobial, calf

2040 Prepartum anti-inflammatory treatment effect on calf type1/type2 immunity ratio using a rapid blood test. R. Sorto^{*1}, Q. Huo², E. Jimenez¹, P. Zarei¹, J. Spring¹, L. Periconi¹, M. Martinez¹, E. Hovingh¹, N. Campos¹, and A. A. Barragan¹, ¹*Department of Veterinary and Biomedical Sciences, Penn State University, State College, PA*, ²*Department of Chemistry, University of Central Florida, Orlando, FL*.

It has been reported that prepartum anti-inflammatory treatment improved immunity status after calving in dairy cows. The objective of this study was to evaluate the effects of dam's acetylsalicylic acid administration 14 d before the expected calving date on calf's type1/type2 immunity ratio during the first 4 weeks after birth using a rapid blood test (D2DX immunity test). Prepartum cows, blocked by body condition score and parity, were randomly allocated to 1 of 2 groups: (1) ASA (80), treated with 4 acetylsalicylic acid boluses (480 grains/bolus; equivalent to 125 g/d); or (2) PLC (80), treated with 4 gelatin capsules filled with water. Singleton female alive calves (CLF) born from this cohort of cows were enrolled in the study (ASA-CLF = 43; PLC-CLF = 41). Blood samples were collected from calves within 3 d after calving and weekly for 4 weeks to assess type1/type2 immunity ratio. The data were analyzed using the UNIVARIATE and MIXED procedures of SAS, as a randomized complete block design. Calves born from cows treated with ASA had a higher type1/type2 immunity ratio within 3 d after birth compared with PLC-CLF (ASA-CLF = 0.079 ± 0.003 ; PLC-CLF = 0.059 ± 0.003 ; $P = 0.0003$). Regardless of treatment, the type1/type2 immunity ratio significantly decreased over time, with the highest ratio observed within 3 d after birth and the lowest at wk 4 (wk 1 = 0.06 ± 0.002 ; wk 2 = 0.04 ± 0.002 ; wk 3 = 0.02 ± 0.002 ; wk 4 = 0.01 ± 0.002 ; $P < 0.0001$). These findings suggest that ASA prepartum treatment may enhance neonatal calf immunity, as evidenced by a higher type1/type2 immunity ratio.

Key Words: prepartum anti-inflammatory treatment, calf immunity, rapid immunity blood test

2041 Association between activity behavior and neonatal calf diarrhea in group-housed dairy calves. A. Welk^{*1}, M. Liefstink^{1,2}, and D. L. Renaud¹, ¹*Department of Population Medicine, University of Guelph, Guelph, ON, Canada*, ²*Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands*.

The objective of this study was to assess how diarrhea status influences activity behavior in the days leading up to and during a diarrhea bout in calves housed in large groups and fed high allowances of milk. Holstein dairy calves were housed in groups of 15 and offered up to 15 L/d of milk replacer by an automated milk feeder. Calves were fecal scored daily for 21 d, and diarrhea was diagnosed when calves had loose feces for ≥ 2 d or watery feces for ≥ 1 d. Each diarrheic calf ($n = 19$ pairs) was paired with a healthy calf, matched for group pen and age of diarrhea onset. Calves were fitted with pedometers from birth to measure activity behaviors, including steps, lying time, and lying bouts. Mixed linear regression models were used to evaluate the association of activity behaviors (from d -3 to d 8 relative to diarrhea diagnosis) and diar-

rhea status, including its interaction with day. Age at onset of diarrhea was included as a fixed effect, day was treated as a repeated measure, and pair was included as a random effect. Similar models evaluated the association between diarrhea status and relative change in activity behaviors from d -3 to d 0, with the baseline activity standardized at 0% on d -3. Overall, diarrheic calves were less active, taking 50% fewer steps than healthy calves from d 0 to 5 ($P = 0.004$). Diarrheic calves also had fewer lying bouts compared with healthy calves (diarrheic: 20.6 vs. healthy: 23.7 ± 0.5 bouts per day, $P < 0.001$) from d -3 to d 8. Lying time was not associated with diarrhea status. For relative change in activity behaviors, a diarrhea status by day interaction was detected on d -1 to d 0, with diarrheic calves having greater reductions in steps compared with healthy calves ($P < 0.001$). Relative change in lying time and lying bouts were not associated with diarrhea status. These results demonstrate that diarrheic calves are less active during a diarrhea bout, and a relative change in steps can be detected 1 d before calves show clinical signs of diarrhea.

Key Words: precision livestock farming, sickness behavior, scours

2043 Computer vision for automated calf respiratory health scoring. B. Xu^{*} and T. von Königslow, *Department of Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY*.

Respiratory diseases significantly impact calf health, welfare, and productivity. Traditional health evaluations rely on subjective human observation, leading to inconsistent diagnoses and scalability challenges in large herds and group housing systems. This study developed and evaluated a computer vision-based framework for automated respiratory health scoring by classifying ear posture, ocular discharge, and nasal discharge using a validated standardized scale. Data were collected from 100 Holstein replacement female dairy calves housed in groups of 20 on a commercial farm in New York. Health scores (0 = normal, 3 = severe) were assigned by 3 trained individuals under veterinary oversight during live observations and validated through image reviews. Facial video keyframes were manually selected, cropped, resized to 640×512 pixels, and annotated using LabelImg. The data set was split into 80% training and 20% testing. The pipeline, including preprocessing, model training, and evaluation, was implemented in Python. The model framework employed a Swin Transformer backbone for multi-scale feature extraction and a deformable transformer query head for classification, supported by a neck module for feature integration. Data augmentation, including random resizing and cropping, were applied to improve generalization. Performance was evaluated using precision, recall, and F1-score and compared with other deep learning-based detection models. The proposed framework outperformed baseline models in classifying respiratory health indicators. For ocular discharge scoring, the model achieved an F1-score of 0.86 with a precision of 0.87 and recall of 0.85. Nasal discharge classification reached the highest F1-score of 0.88, with a precision of 0.86 and a recall of 0.89. Ear posture classification yielded an F1-score of 0.82 with a precision of 0.75 and a recall of 0.90. The findings demonstrate the potential of the proposed framework as an objective and scalable solution for respiratory health assessment in calves. By facilitating early disease detection, it contributes to improved livestock health monitoring and management.

Key Words: respiratory disease, health scoring, computer vision

2044 The effect of bovine leukemia virus infection on dairy calf health and growth. K. Broadfoot^{*1}, F. van der Meer², R. Couto Serrenho¹, F. C. Pharo¹, A. Keunen³, and D. L. Renaud¹, ¹*Department of Population Medicine, Ontario Veterinary College, Guelph, ON*,

Canada, ²Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada, ³Mapleview Agri Ltd., Palmerston, ON, Canada.

The objective of this cohort study was to investigate whether bovine leukemia virus (BLV) positivity in dairy calves is associated with health and growth outcomes. The study was conducted at a commercial calf raising facility in Southwestern Ontario, Canada from May 2023 to May 2024 and included 768 calves (726 Holstein, 42 crossbred) aged approximately 3 to 10 d at arrival. Whole blood samples were collected on d 1, 30, and 84 after arrival, and DNA was extracted to determine BLV proviral load via BLV SS1 qPCR assay (CentralStar Cooperative Inc., East Lansing, MI). Calves were scored twice daily for fecal consistency and respiratory disease using the UC Davis scoring chart. They were also weighed at arrival and then weekly until departure (84 d). Average daily gain (kg/d) and feed efficiency (ME/kg of gain) were assessed using mixed linear regression models, and a Poisson and negative binomial model analyzed the frequency of observations with respiratory scores >5 and fecal consistency scores >2, respectively. A zero-truncated Poisson model analyzed observations with no clinical signs of respiratory disease (score = 0), and treatment for respiratory disease and diarrhea was assessed using Cox proportional hazards models. Two calves were excluded due to incomplete blood sampling, leaving 766 for analysis. Of the 766 calves, 43 calves (5.6%) tested positive for BLV over 84 d. The BLV-positive calves had greater risk of respiratory scores >5 (IRR = 1.91, 95% CI: 1.05–1.32, $P < 0.001$) and a lower risk of respiratory scores of 0 (IRR = 0.98, 95% CI: 0.95–1.00, $P = 0.007$). No significant difference was found between BLV positivity and average daily gain, feed efficiency, or diarrhea. The study found that BLV positivity was associated with an increased risk of respiratory disease and a reduced likelihood of having no clinical signs of respiratory disease. These findings suggest that BLV may contribute to respiratory disease pathogenesis, but further research is needed to clarify its impact on overall calf health.

Key Words: bovine leukemia virus, calf health, retrovirus

2045 Supplementation of naturally sourced caffeine, from green tea extract, on vigor, health, and lying behavior of Holstein heifer calves. K. M. R. Lutz¹, D. R. Renaud², M. A. Steele¹, and T. J. DeVries^{*1}, ¹Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, ²Department of Population Medicine, University of Guelph, Guelph, ON, Canada.

The objective of this study was to determine how different doses of green tea extract (a natural caffeine source) affect the vigor, health, and behavior of dairy calves until 28 d of life. Holstein heifer calves were assigned to 1 of 3 treatments: (1) single (SNG; $n = 42$) 15-mL dose of green tea extract (327.6 mg caffeine; Calf Perk, TechMix, LLC), (2) 30-mL double dose (DBL; $n = 39$; 655.2 mg caffeine), or (3) control (CON; $n = 39$; 15-mL placebo). Treatment was given orally before colostrum (within 2 h of life, 4 L of colostrum replacer, 300 g of IgG). Blood was sampled at 0, 2, 6, 24, 48, and 72 h of life for serum IgG and analyzed by radial immunodiffusion. Vigor scoring occurred at 0, 2, 24, 48, and 72 h of life (with scoring of up to 27 points, where higher is better; Calf VIGOR Scorer app, University of Wisconsin–Madison), and health scoring occurred at 24 and 72 h, and twice weekly until 28 d of life (with scoring of up to 30 points, where lower is better; Calf Health Scorer app, University of Wisconsin–Madison). Lying behavior monitors (HOB0 data loggers) were placed on the hind leg of each calf after colostrum feeding and replaced weekly for data downloading until 28 d of life. Data were analyzed using mixed-effect, repeated measures, and linear regression models. When an overall effect of treatment was detected, differences between individual treatment calves and CON

calves were made by pairwise comparisons. Average serum IgG of SNG calves (14.2 ± 0.24 mg/mL) was higher ($P = 0.01$) than CON calves (13.8 ± 0.24 mg/mL), whereas DBL calves (13.9 ± 0.24 mg/mL) did not differ ($P = 0.34$) from CON calves. Vigor scores were greater ($P = 0.04$) for DBL calves (23.7 ± 0.22) compared with CON calves (23.1 ± 0.23), whereas SNG calves (23.1 ± 0.23) did not differ ($P = 0.96$) from CON calves. Health scores for SNG calves (4.5 ± 0.14) did not differ ($P = 0.38$) from CON calves (4.7 ± 0.14), but DBL calves (4.3 ± 0.14) had improved health scores compared with CON calves ($P = 0.03$). Both DBL (1281.4 ± 20.7 min/d) and SNG ($1,294.2 \pm 20.6$ min/d) calves had lesser ($P < 0.001$) lying time on d 1 compared with CON calves ($1,384.5 \pm 20.6$ min/d), which may be linked to increased vigor. Single supplementation of caffeine via green tea extract improved IgG absorption, and a DBL dose improved overall vigor and health scores. Both doses had reduced lying time (increased activity) on d 1 following supplementation. Thus, green tea extract supplementation could be particularly impactful for calves who have experienced low vigor due to birth complications.

Key Words: caffeine, calf health, lying behavior

2046 Detection of influenza A nucleoprotein antigen and H5-specific antibodies in dairy cow milk samples. L. R. Ahlers^{*1}, N. Sammons¹, B. S. Lane¹, A. Mathew¹, S. Misaghian¹, I. Shittu², G. C. Gray², G. Sigal¹, and J. N. Wohlstadter¹, ¹Meso Scale Diagnostics, Rockville, MD, ²University of Texas Medical Branch, Galveston, TX.

The highly pathogenic avian influenza (HPAI) H5N1 (clade 2.3.4.4b) is of growing concern to both human and animal health. Infections are currently considered widespread in dairy herds and sporadic in poultry farms. The concern is growing, as additional influenza H5 subtypes besides the B3.13 genotype from the initial spillover event to cows are being identified. New high-throughput species-independent assays are needed to detect H5 from a variety of species and sample types, including bovine serum and milk. We developed high-throughput plate-based immunoassays to measure influenza A nucleoprotein (NP) antigen and H5 hemagglutinin (HA) specific antibodies. Both assays are species-agnostic and use electrochemiluminescence (ECL) technology from Meso Scale Discovery to perform highly specific and sensitive measurements. The influenza A NP assay is a sandwich immunoassay for antigen detection. Using this assay, we detected NP antigen in 26 of 50 cow milk samples tested from H5N1-exposed herds, with concentrations ranging from 14 to 486 $\mu\text{g/mL}$. We measured H5 HA-specific antibodies with a bridging serology assay, in which we incubate antibody-containing samples with labeled constructs of the H5 HA head domain. This assay is calibrated with polyclonal serum from rabbits immunized with H5 HA antigen, and antibody concentrations are presented in arbitrary units (AU). We measured H5-specific antibodies in 11 of 50 cow milk samples from H5N1-exposed herds. We did not detect NP antigen or H5-specific antibodies in milk samples from a healthy, unexposed herd. The antigen and antibody levels are complementary. Together the assays identify 64% of the H5N1-exposed milk samples as containing NP antigen, H5 antibodies, or both. This is greater than the number of milk samples that tested positive by qRT-PCR (46%). These assays can screen samples from any host species for influenza A antigen and antibodies against H5 HA.

Key Words: H5N1, antigen detection, immunoassay, serology

2047 Effects of wildfire PM_{2.5} on calf lung and immune function. D. Sarantopoulos^{*1}, J. Cruickshank², S. Lepiane², M. Kamyabi¹, A. Pace¹, L. De Moura Pereira¹, J. Ranches², D. Konetchy¹, P.

Rezamand^{1,3}, and A. L. Skibieli¹, ¹University of Idaho, Moscow, ID, ²Oregon State University, Corvallis, OR, ³University of Connecticut, Storrs, CT.

Wildfire particulate matter (PM_{2.5}) affects calf health, but underlying mechanisms are unknown. This prospective cohort study assessed impacts of PM_{2.5} on calf lung and immune function. Holstein heifer calves with matched nutrition and management were exposed to PM_{2.5} (WF, n = 20, Moscow, ID) or unexposed (CON, n = 18, Corvallis, OR). Daily temperature-humidity index (THI) and PM_{2.5} concentrations were recorded. At 3, 9, and 12 wk of age, lung ultrasound and health scores and lung function values (tidal volume [VT], minute ventilation [VE]) were recorded. Blood was collected for hematology, granulocyte respiratory burst (RB), and acute phase protein analyses. Trans-tracheal wash fluid (TW) was collected at 3 and 12 wk for immunophenotyping. Data were analyzed with general or generalized linear mixed models, with fixed effects of group, age, and their interaction and repeated effects of age within calf or random effect of calf. The WF calves were exposed to 24 d of moderate PM_{2.5} ($\leq 41.8 \mu\text{g}/\text{m}^3$) from 3 to 9 wk. In the CON group, PM_{2.5} was low ($\leq 8.9 \mu\text{g}/\text{m}^3$). The THI did not differ between groups. At 3 wk, WF had higher TW granulocyte percent ($63.83\% \pm 3.22\%$ vs. $48.95\% \pm 3.53\%$) but lower RB ($42.96\% \pm 3.14\%$ vs. $65.62\% \pm 3.40\%$) compared with CON (both $P \leq 0.004$). At 9 wk, WF had greater serum albumin (1.67 ± 0.04 vs. $1.52 \pm 0.04 \text{ g/dL}$) and haptoglobin (0.42 ± 0.02 vs. $0.34 \pm 0.02 \text{ mg/mL}$) than CON (both $P < 0.02$). At 12 wk, WF had higher haptoglobin (0.40 ± 0.02 vs. $0.31 \pm 0.02 \text{ mg/mL}$), odds of lung consolidation (odds ratio: 17.68, 95% CI: 3.61–86.68), and coughing (odds ratio: 19.25, 95% CI: 1.57–236.07), but lower VT (0.82 ± 0.04 vs. $1.04 \pm 0.04 \text{ L}$) and TW percent CD163+ (1.25 ± 0.34 vs. $3.40 \pm 0.97\%$) and CD163+/CD68+ macrophages (6.77 ± 2.64 vs. $29.45 \pm 2.78\%$) than CON (all $P < 0.03$). The WF group had higher VE (21.89 ± 0.85 vs. $11.99 \pm 0.49 \text{ L/min}$) and serum amyloid A ($241,228.53 \pm 19,927.89$ vs. $171,699.31 \pm 14,951.58 \text{ ng/mL}$), but lower white blood cells (8.01 ± 0.22 vs. $8.80 \pm 0.25 \times 10^9 \text{ cells/L}$), lymphocytes (4.82 ± 0.18 vs. $6.28 \pm 0.18 \times 10^9 \text{ cells/L}$), monocytes (0.16 ± 0.01 vs. $0.70 \pm 0.06 \times 10^9 \text{ cells/L}$), eosinophils (0.05 ± 0.01 vs. $0.10 \pm 0.01 \times 10^9 \text{ cells/L}$), and basophils (0.02 ± 0.002 vs. $0.03 \pm 0.003 \times 10^9 \text{ cells/L}$) than CON (all $P < 0.03$). We conclude that PM_{2.5} exposure alters calf lung function and local and systemic immune responses.

Key Words: air quality, pulmonary, inflammation

2048 Assessing the health-supportive effects of *Bacillus* spp. under an in vitro assay. L. C. Capern, R. Gresse, O. C. M. Queiroz, and B. I. Cappellozza*, Novonosis, Lyngby, Zealand, Denmark.

Per definition, direct-fed microbials (DFM) should confer health benefits to the host, when offered in the correct dosage. Previous authors have reported a wide, although not specific, range by which bacteria-based DFM support the health of livestock, including the inhibition of potentially harmful bacteria, such as *Salmonella enterica* and *Campylobacter jejuni*. Hence, we evaluated the effects of *Bacillus* spp. on the in vitro growth of *S. enterica* and *C. jejuni*. In experiment (Exp.) 1, *Bacillus licheniformis* 809 (BL), *Bacillus subtilis* 810 (BS), or the combination of both (CMB), were tested against *S. enterica* Typhimurium (CHCC 18909) for a 24-h period. In Exp. 2, CMB was tested against *C. jejuni* (CHCC 30799) for a 32-h period. For both experiments, on d 1, the DFM were inoculated at $5 \times 10^5 \text{ cfu/g}$ feed, and samples were incubated aerobically at 37°C under 120 rpm shaking for 18 h. The *S. enterica* Typhimurium was inoculated on trypticase soy agar (TSA) and incubated aerobically at 37°C for 18 to 24 h, whereas *C. jejuni* was inoculated on TSA + sheep blood and incubated microaerophilic at 42°C for 18 to 24 h. On d 2, the pathogens were inoculated in all feed samples to a

concentration of 1×10^5 or 10^6 cfu/g feed for Exp. 1 and 2, respectively, and all samples were incubated aerobically at 37°C, 120 rpm shaking, for 24 h (Exp. 1) or microaerophilic at 42°C for 32 h (Exp. 2). Aliquots were collected at selected time points post-incubation and subsequently diluted and enumerated on selective agar (MacConkey, Oxoid, in Exp. 1; *Campylobacter* agar, SSI, in Exp. 2) in duplicates. All data were analyzed with SAS (version 9.4), and significance was set at $P \leq 0.05$. Adding *Bacillus* spp., regardless of the combination, reduced the counts of *S. enterica* Typhimurium vs. CON at 4 (6.63, 5.36, 5.63, and 5.68 log₁₀ cfu/g feed for CON, BL, BS, and CMB, respectively), 8 (9.40, 5.83, 7.07, and 7.03 log₁₀ cfu/g feed for CON, BL, BS, and CMB, respectively), and 24 h (10.5, 7.00, 8.23, and 8.35 log₁₀ cfu/g feed for CON, BL, BS, and CMB, respectively; $P < 0.001$), whereas BL also resulted in lower counts when compared with BS and CMB at 8 and 24 h ($P < 0.0001$). In Exp. 2, a treatment $\times$ hour interaction was observed for the counts of *C. jejuni* ($P < 0.01$), as CMB inoculation yielded lower counts when compared with CON at 24 (7.76 vs. 4.78 log₁₀ cfu/g for CON and CMB, respectively) and 32 h (8.81 vs. 4.88 log₁₀ cfu/g for CON and CMB, respectively; $P < 0.001$). In summary, *Bacillus* spp. were effective in reducing the counts of *Salmonella enterica* Typhimurium and *Campylobacter jejuni*, 2 potentially harmful bacteria and of importance to cattle, under an in vitro methodology.

Key Words: *Bacillus* spp., *Campylobacter jejuni*, *Salmonella enterica* Typhimurium

2232 Insights from a single outbreak: Cow-level risk factors associated with HPAI in lactating Holstein cows. A. Rico^{*1,2}, N. Blaine³, A. Lago⁴, and N. Silva-del-Rio^{1,2}, ¹Department of Population Health and Reproduction, University of California–Davis School of Veterinary Medicine, Davis, CA, ²Veterinary Medicine Teaching and Research Center, University of California–Davis School of Veterinary Medicine, Davis, CA, ³Jager Ag, LLC, Eaton, CO, ⁴DairyExperts Inc., Tulare, CA.

Highly pathogenic avian influenza (HPAI) H5N1 (clade 2.3.4.4b) has spilled into dairy cattle populations. Seven months after the initial outbreak, many unknowns remain. This study aims to identify cow-level factors (e.g., days in milk [DIM], milk yield, parity, pregnancy) that may increase susceptibility to H5N1 infection using on-farm records. We enrolled 3,281 Holstein lactating cows from a commercial herd with 12 pens, mainly housing pregnant cows from a sister facility. The outbreak peaked in late May. Cow information, including DIM, milk yield, parity, reproductive status, and pen allocation, was extracted from herd records as of May 1, 2024. Cases were defined as cows with “flu” recorded in their health history by August 31, 2024. Comparable pens were identified via cluster analysis. Associations between cow-level factors and case risk were assessed using chi-square and *t*-tests. A 1:1 matched cohort (98 pregnant, 98 non-pregnant) was used to analyze pregnancy effects. A nested case-control study was analyzed using conditional logistic regression, while a cohort-based case-control study (pregnant cows in comparable pens) used mixed-effects logistic regression with pen as a random variable. The significance level was 0.05. The overall case risk was 16.2%. In the descriptive study (n = 3281), DIM, milk yield, and days carrying calf were not significantly different between cases and non-cases ($P > 0.05$), while pregnancy, parity, and pen allocation were ($P < 0.05$). Risk varied across pens (7–27%) and among comparable pens (7%–24%). In the second study (n = 196), non-pregnant cows had a 4% risk, while pregnant cows had an 18% risk (OR = 4.9; 95% CI: 1.6, 14.9). In the final study (n = 1,546), only parity was a significant risk factor; first-parity cows had a 9% risk, while multiparous cows had an 18% risk (OR = 2.1; 95% CI: 1.5, 2.8). Key findings: (1) similar pens

had varying risk levels, (2) pregnant lactating cows had higher case risk than non-pregnant ones, and (3) among pregnant cows, first-parity cows had lower risk than multiparous cows.

Key Words: H5N1, avian influenza, highly pathogenic avian influenza (HPAI)

2233 Multiple gastrointestinal microbial virulence genes are increased in rectal swab samples from lactating dairy cows of HPAI-positive farms. S. R. Fensterseifer^{*1}, A. M. Lange², E. A. Galbraith², R. P. Arias¹, S. Busato¹, S. Son², and M. R. King², ¹United Animal Health Inc., Sheridan, IN, ²Microbial Discovery Group, Oak Creek, WI.

Highly pathogenic avian influenza A (HPAI H5N1) virus has been detected in dairy cows in the United States since March 2024. Although the mortality rate has been low in cows compared with its devastating effects in poultry, the full impact of HPAI infections on cow health remains unclear. Coinfections with multiple microbes and increased pathogen loads have been shown to exacerbate disease severity, contribute to complications, and affect viral shedding and replication. This study aimed to investigate if lactating dairy cows from HPAI-positive (HPAI+) and non-positive (NoHPAI) commercial dairy farms had any differences in key microbial virulence genes associated with gastrointestinal (GI) disease. Rectal swab samples were taken from lactating dairy cows in 4 HPAI+ farms within 1 mo of infection ($n = 113$ cows, 2.88 ± 0.13 lactations, 162.9 ± 10.3 DIM) and compared with 19 NoHPAI farms ($n = 571$ cows, 2.51 ± 0.06 lactations, 110.4 ± 4.2 DIM) in similar geographical regions. A qPCR panel was used to quantify common GI microbial virulence and marker genes. Kruskal–Wallis, followed by Mann–Whitney pairwise comparisons, and chi-squared tests were performed to detect differences in gene quantity and frequency, respectively, between HPAI+ and NoHPAI cows. Cows from HPAI+ farms had increased ($P < 0.001$) quantities of total *E. coli* ZnT and *E. coli* marker O157, as well as the *E. coli* virulence genes *eaeA* ($P = 0.01$), enteroaggregative stable toxin *EAST1* ($P < 0.001$), and Shiga toxin 1 ($P = 0.016$) compared with cows from NoHPAI farms. *Clostridium perfringens* α toxin gene *cpa* and *Salmonella* were also higher ($P < 0.001$) in cows from HPAI+ farms compared with NoHPAI. *Eimeria bovis* and *Aspergillus flavus* were detected in rectal swabs from HPAI+ and NoHPAI cows, but no differences ($P \geq 0.929$) in gene quantities were found. In conclusion, cows from HPAI+ farms had increased GI loads of pathogenic *E. coli*, *C. perfringens*, and *Salmonella*, which may elevate disease risk and exacerbate existing health issues. Insight into HPAI coinfection highlights the need for further research to develop strategies that promote cow health during outbreaks.

Key Words: coinfection, bird flu, H5N1

2234 Effects of highly pathogenic avian influenza H5N1 infections on dairy operations. D. R. Bruno^{*1}, C. Stenkamp-Strah², B. Melody³, R. G. S. Bruno⁴, and J. Lombard², ¹University of California Agriculture and Natural Resources, Cooperative Extension Fresno County, Fresno, CA, ²College of Veterinary Medicine and Biomedical Sciences, AgNext, Colorado State University, Fort Collins, CO, ³Lander Veterinary Clinic, Turlock, CA, ⁴Zoetis Inc., Parsippany, NJ.

In 2024, highly pathogenic avian influenza (HPAI) H5N1 was detected for the first time in US dairy cattle in Texas. H5N1 has now been confirmed in dairy herds in 17 states. This study examined the impact an H5N1 outbreak has on herd health and productivity, specifically evaluating milk production, disease duration, and herd removal, utilizing records from affected Jersey and Holstein herds in California, Colorado,

and Michigan. Farm data from these herds, including treatment records, were retrieved from DC305. Herd sizes ranged from 1,000 to 6,000 cows. During the outbreak, farm employees administered treatment to cows displaying clinical signs of H5N1, including abnormal milk, reduced feed intake, decreased rumination, mild respiratory symptoms (e.g., clear nasal discharge, labored breathing), lethargy, dehydration, and altered stool consistency. Descriptive statistical analysis was used to summarize herd-level disease patterns, treatment frequencies, mortality rates, and production losses. Records show that approximately 60% of cows exhibiting clinical signs were treated only once, while the remaining affected cows received multiple treatments. The highest treatment rate occurred between d 6–8 after the first cows displaying clinical signs were treated. Based on treatment records, disease duration at the herd level lasted between 10 and 45 d. Mortality rates varied by farm, with H5N1-related herd removal ranging from 0% to 15%, depending on herd size. Affected dairies experienced a 10% to 50% decline in milk production (10 to 45 lb per cow), and a minimal to 3-fold increase in bulk tank somatic cell counts compared with pre-outbreak levels. Subsequent lactation milk production levels are being evaluated as the H5N1-affected cows reach this point in time. These findings underscore the substantial impact of H5N1 on dairy herd health and productivity. Early detection, intervention, and preventive management strategies are critical for mitigating the disease's effects and maintaining herd sustainability.

Key Words: highly pathogenic avian influenza (HPAI), dairy, cow

2240 Influenza A virus sialic acid receptor distribution in the bovine mammary gland across physiological states. P. H. Baker^{*1}, L. M. Glendenning², M. X. S. Oliveira³, B. A. Cobb², B. D. Enger³, and S. N. Langel¹, ¹Center for Global Health and Diseases, Department of Pathology, Case Western Reserve University, Cleveland, OH, ²Department of Pathology, Case Western Reserve University, Cleveland, OH, ³Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH.

Highly pathogenic avian influenza (HPAI) A (H5N1) virus emerged in lactating dairy cattle in March 2024, causing mastitis, economic losses, and infections in other farm animals and workers. Influenza A virus (IAV) initiates infection by binding to terminal sialic acid (SA) residues on oligosaccharide chains of host cell surface glycoproteins. Human-adapted IAV strains typically recognize $\alpha 2,6$ -linked SAs, which are predominant in the upper respiratory tract, while avian IAV strains preferentially bind $\alpha 2,3$ -linked SAs, commonly found in the intestinal tract of birds and the lower respiratory tract of humans. Recently, both $\alpha 2,6$ - and $\alpha 2,3$ -linked SAs were found to be expressed in the mammary glands of lactating cows; however, little is known about their distribution across different physiological states. Using lectin histochemistry, we compared $\alpha 2,6$ - and $\alpha 2,3$ -linked SA distribution in the mammary glands of prepubertal dairy calves, pregnant dairy heifers (6.5- and 8.5-mo gestation), and lactating cows. Each group expressed both SA $\alpha 2,6$, the preferred receptor for human and swine IAVs, and importantly, SA $\alpha 2,3$, which is favored by avian IAVs. Notably, mammary glands of pregnant heifers exhibited the highest expression of SA $\alpha 2,3$, with predominant expression within the alveolar lumens. These findings suggest that SA $\alpha 2,3$ expression may vary with reproductive life stage, raising the possibility that pregnant heifers may be susceptible to H5N1-induced mastitis and could serve as a viral reservoir before entering the lactating herd. Future studies will assess the susceptibility of mammary epithelial cells from pregnant heifers to H5N1 and examine whether their secretions interact with the virus in ways that promote or inhibit infection.

Key Words: avian influenza, H5N1, mammary gland, sialic acid receptor

Breeding and Genetics 1: Posters

2049 Inbreeding depression for milk yield under heat stress in Italian Holstein cows. F. Tiezzi¹, C. Maltecca², J. C. C. Panetto^{*1,5}, J.-T. van Kaam⁴, M. Cassandro⁴, C. Cipolat-Gotet³, M. Ablondi³, A. Zanotti³, and R. Finocchiaro⁴, ¹*Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, Florence, Italy*, ²*Department of Animal Science, North Carolina State University, Raleigh, NC*, ³*Department of Veterinary Science, University of Parma, Parma, Italy*, ⁴*Associazione Nazionale Allevatori della Razza Frisone, Bruna e Jersey Italiana (ANAFIBJ), Cremona, Italy*, ⁵*Embrapa Dairy Cattle, Juiz de Fora, Brazil*.

This study aimed at assessing inbreeding depression for milk yield under heat stress in Italian Holstein cows. A total of 414,514 milk yield records were available for 24,395 cows, raised on 703 herds, which were visited on 25,001 herd-test days. For each herd-test date, the average temperature, humidity, and temperature-humidity index (THI) for the week before the visit was available. Milk yield averaged 37.9 kg/d (± 8.92 kg/d). Genomic inbreeding estimates were assessed via 3 methods: F_{grm}, derived from the diagonal of the genomic relationship matrix (GRM); F_{roh}, calculated as the ratio of the sum of runs of homozygosity (ROH) segments to the entire genome; and F_{roh}_class, which categorizes ROH segments into 6 classes based on their sizes. A first mixed model was implemented to assess the impact of environmental stress on milk yield. Heat stress-related covariates (temperature, relative humidity, or THI) were fitted in the model as cross-classified effects with 5 levels, with relative humidity resulting in the strongest impact on milk yield. A second model was implemented to assess the impact of inbreeding depending on environmental conditions. This model included the fixed effects of lactation stage by lactation number (30 classes), relative humidity (5 classes), and inbreeding covariate nested within relative humidity class. Additionally, herd-test date and cow permanent environment and additive genetic random effects were fitted. Results show that cows had higher milk yield under low, medium-low, and medium humidity classes, and yield decreased with humidity increasing to medium-high or high. Inbreeding depression, regardless of its metric, was strong and statistically significant at the high relative humidity class ($>84.3\%$) as well as at the low relative humidity class ($<60.7\%$). In contrast, depression was not statistically significant for the medium class and weak for the medium-low and medium-high classes. Further research will focus on additional productive traits and their inbreeding depression under heat stress.

Key Words: dairy cattle, genome, heat tolerance

2050 Genomic analysis of differential somatic cell count in Valle del Belice sheep. S. Mastrangelo¹, S. Riggio¹, C. Persichilli², M. Congiu³, G. Senczuk², and A. Cesarani^{*3}, ¹*Dipartimento Scienze Agrarie, Alimentari e Forestali, University of Palermo, Palermo, Italy*, ²*Dipartimento Agricoltura, Ambiente e Alimenti, University of Molise, Campobasso, Italy*, ³*Dipartimento di Agraria, University of Sassari, Sassari, Italy*.

Somatic cell count (SCC) is the most commonly used measure of udder health in dairy animals. Elevated SCC values are often associated with subclinical mastitis, which in turn affects milk quality and its ability to coagulate. However, SCC alone does not provide information on the distribution of the different cell types. Therefore, differential somatic cell count (DSCC)—a parameter that combines the proportions of polymorphonuclear leukocytes and lymphocytes—has been proposed

as a more accurate indicator of mastitis in cows and sheep. Currently, little information on the genomic aspects of DSCC is available in sheep. The aim of this study was to estimate the heritability of DSCC and compute a genome-wide association study (GWAS) of DSCC in Valle del Belice dairy sheep breed. A total of 337 ewes (from 9 farms) were considered. One milk sample from each ewe was collected to analyze composition and DSCC. All animals were genotyped with 39,845 SNPs after quality control. The values of DSCC were analyzed with an animal model that included animal and farm as random effects, parity as cross-classified fixed effect, and day in milk as fixed covariate. The animal effect was modeled using the genomic relationship matrix. The variance component estimation and the GWAS were carried out using the blupf90 suite. Genomic breeding values were estimated for all animals and then back-solved to get SNP effects and their *P*-values. The estimated heritability was 0.10 ± 0.07 . A total of 1,574 markers were significant, but, after the Bonferroni correction at chromosome level, only 1 SNP (rs414000671, on chromosome 9 at 76,801,456 base pairs) was found to be significantly associated with DSCC. The SNP mapped within the *ANKRD46* gene and close (± 250 kb) to *PABPC1*, *SNX31*, and *RNF19A*. Two genes (*ANKRD46* and *SNX3*) have been associated with susceptibility to infectious bovine keratoconjunctivitis in Hereford cattle. The *RNF19A* gene, also known as Dorfin, plays a role in the innate immune system in response. Even if characterized by a low heritability, these preliminary results suggest the possibility of including DSCC as a breeding goal in the Valle del Belice breed. The study was supported by the GEREMES project (CUP J53D23013860001; Finanziamento dell'Unione Europea – NextGenerationEU – missione 4, componente 2, investimento 1.1).

Key Words: udder health, genomic selection, GWAS

2051 Update on genetic parameters and introduction of new female fertility traits in the US national evaluation. J. Graham^{*1}, T. McWhorter², J. Cole^{2,3,4}, A. Legarra², A. M. Miles¹, G. Dutta⁵, B. Fragomeni⁵, and P. M. VanRaden¹, ¹*USDA-AGIL, Beltsville, MD*, ²*Council on Dairy Cattle Breeding, Bowie, MD*, ³*Department of Animal Sciences, Donald Henry Barron Reproductive and Perinatal Biology Research Program, and the Genetics Institute, University of Florida, Gainesville, FL*, ⁴*Department of Animal Science, North Carolina State University, Raleigh, NC*, ⁵*University of Connecticut, Storrs, CT*.

The genetic evaluation of female fertility traits in dairy cattle has progressed over 2 decades, integrating 4 fertility traits into the national evaluation so far. However, concerns have arisen over the extension of the voluntary waiting period (VWP) due to longer lactation cycles. Current DPR calculations assume a fixed 50-d VWP, which may not reflect modern reproductive strategies. This study re-estimates genetic parameters for 4 fertility traits—daughter pregnancy rate (DPR), cow conception rate (CCR), early first calving (EFC), and heifer conception rate (HCR)—across all breeds in the national database. Additionally, a new trait, first service to conception (FSC), is introduced along with a revised DPR formula. Records from the National Cooperator Database, covering 5 major dairy breeds (2003–2023), were used. Cows calving before 2003 were excluded due to incomplete CCR and FSC data. Preliminary findings suggest FSC enhances fertility evaluations, providing a more comprehensive reproductive assessment independent of VWP. A tailored approach calculated an adjusted DPR (DPR_{adj}) to account for herd VWP variability. The mean DPR value was 47.79%, whereas

DPR_{adj} increased it to 57.72%. The mean FSC was 62.64 d. The genetic correlations of FSC with other fertility traits were 0.97 with CCR and DPR, 0.40 with HCR, and 0.32 with EFC. Heritability estimates were low but more than twice previous estimates, with DPR and FSC at 0.035, up from 0.016. Heritabilities for each breed and trait will be presented. Predicted transmitting abilities (PTA) were calculated to assess trait inclusion or replacement in the index. The highest correlation among the top 10% of bulls occurred when replacing DPR with DPR_{adj} (0.99). Adding FSC to the original model reduced the correlation to 0.88, which indicates slight re-ranking of top bulls. Among animals with PTA reliability above 50%, the lowest correlation was between the original index and the index replacing DPR with FSC (0.95). Next steps involve integrating FSC and DPR_{adj} into the national evaluation and further refining genetic predictions. This work advances genetic evaluation in dairy cattle, improving reproductive efficiency and productivity.

Key Words: fertility, evaluation, genetics

2052 Analysis of gene sets enriched with respiratory disease in Holstein calves. A. L. Herrick^{*1}, J. N. Kiser^{1,2}, and H. L. Neibergs¹, ¹Department of Animal Sciences, Washington State University, Pullman, WA, ²Washington Animal Disease Diagnostics Laboratory, Pullman, WA.

Bovine respiratory disease (BRD) is a multifactorial disease that remains a leading cause of morbidity and mortality in the dairy industry. The objective of this study was to identify genomic regions associated with BRD in pre- and post-weaning calves using a gene set enrichment analysis-SNP (GSEA-SNP). All study animals were from a single dairy. Phenotypes were based on farm records of BRD treatment. Animals affected by BRD were divided into 2 groups: 520 pre-weaning calves that were up to 60 d of age, and 2,016 post-weaning calves that were 61–421 d of age. Controls consisted of 3,681 pre-weaning healthy calves that were maintained in the herd at least 60 d and 3,212 healthy post-weaning calves that were maintained in the herd at least 421 d and that never had a recorded BRD treatment or diagnosis from birth. Genotyping was performed with Zoetis CLARIFIDE Plus and then imputed to approximately 778,000 SNPs using BEAGLE 4.1. The imputation reference population consisted of 4,800 Holsteins that were genotyped using Illumina BovineHD BeadChip. The GSEA-SNP was performed in R (v. 3.6.3), using the GenGen package. The SNP were mapped to 21,039 protein coding genes within 17 kb based on the ARS-UCD 1.2 genome assembly. Gene sets from Bio Carta, GO, KEGG, PANTHER, and Reactome were used with a normalized enrichment score (NES) threshold of NES ≥ 3 for enrichment based on phenotype-based permutation in GenABEL in R. The Reactome gene set, Phospholipid Metabolism, was enriched (NES ≥ 3) with pre-weaning BRD and consisted of 86 leading edge genes. Two Reactome and 5 GO gene sets were enriched (NES ≥ 3) for BRD in post-weaning calves and consisted of a total of 162 unique leading-edge genes. The leading-edge gene *COL4A3BP* was enriched for BRD in both pre- and post-weaning calves. The gene sets and leading-edge genes enriched for BRD had common functions involving immune cell development, cellular communication, and disease response and inflammation and can be used to further understand the disease mechanisms associated with enhanced resistance to BRD.

Key Words: bovine respiratory disease (BRD), gene set enrichment analysis-SNP (GSEA-SNP), calf

2053 Genetic analysis of lactation consistency in US Holsteins using temporal variation in milk production. P. Khanal^{*}, P. Ross, and N. Deeb, *STgenetics*.

Consistency in daily milk production is essential for herd management, feed allocation, and milking efficiency in dairy operations, yet its genetic basis remains underexplored. This study aimed (1) to estimate genetic parameters for lactation consistency, defined as temporal variance of milk yield (TVAR) across lactation stages in US Holsteins, and (2) to assess correlations between genomic predicted transmitting abilities (GPTAs) of TVAR and economically important traits in the Net Merit index (NMS). A total of 1,062,405 daily milk yield records from 50 to 305 d in milk (DIM) were collected from 5,185 primiparous cows at the STgenetics research herd (2019–2024) using an automatic milking system. The TVAR was computed as the log-transformed variance of daily deviations between observed and expected milk yields at 50–150 DIM and 151–305 DIM, using locally estimated scatterplot smoothing with a 0.75 span and polynomial quantile regression using 0.7 quantile. Analyses were conducted using mixed model equations, with herd-year-season as fixed effects, age at calving as a covariate, and additive genetic effects as random. Variance components were estimated using pedigree information, and GPTAs were derived from genomic relationships. Heritability estimates ($\pm$ SE) of TVAR ranged from 0.28 ± 0.03 to 0.31 ± 0.03 , indicating a significant genetic influence on lactation consistency. A high genetic correlation (>0.9) between lactation stages suggests that early-lactation variability predicts overall consistency, enabling selection based on partial records. Moderate to high correlations were observed between TVAR-GPTAs and GPTAs for milk yield (0.52), fat yield (0.23), and protein yield (0.40), indicating that higher-yielding cows exhibit greater daily variability. The TVAR also correlated favorably with some health traits but showed no genetic link to feed efficiency traits such as feed saved and EcoFeed. These findings highlight a trade-off between lactation consistency and milk yield, where lower variability is associated with better health outcomes but unfavorably correlated with higher production levels. Balancing these traits in breeding programs is crucial for optimizing herd performance.

Key Words: lactation consistency, resilience, genomic selection

2054 Seasonal impacts on bovine milk and feces microbiome diversity. A. M. Miles¹, J. Lourenco², and B. Fragomeni^{*3}, ¹Animal Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD, ²Department of Animal and Dairy Science, University of Georgia Athens, Athens, GA, ³Department of Animal Science, University of Connecticut, Storrs, CT.

Heat stress significantly impacts animal welfare, increases disease incidence, alters milk composition, and reduces reproductive performance. Most research has focused on physiological or behavioral metrics of heat stress, and there has been limited investigation into the molecular mechanisms underlying heat stress. The aims of this proof-of-concept study were to demonstrate that dairy cow microbiomes are impacted by heat stress and that this can be measured by sampling cows in periods of heat stress and in thermoneutral conditions. A cohort of 20 cows at the University of Connecticut Kellogg Dairy Center were followed through spring (representing pre-heat stress), summer (heat stress), and fall (post-heat stress), in 2023. Cow feces and milk were sampled in each season and initially characterized by 16S rRNA gene profiling on the Illumina platform. Seasonal impacts on microbiome diversity were observed in both milk and feces. Concerning milk, Pielou's evenness was higher in fall than in spring or summer ($P < 0.01$), and measures of Faith's phylogenetic diversity suggest that spring may be less diverse than summer or fall ($P \sim 0.10$). Bray-Curtis and weighted Unifrac distances indicate that spring and summer microbial composition are highly similar, but fall is divergent from both ($P < 0.05$). Concerning feces, Pielou's evenness did not differ between fall and summer, but spring

evenness was lower than both ($P = 0.02$ and $P < 0.01$, respectively). Analysis of Faith's phylogenetic diversity suggested fecal microbial composition in the spring is much less diverse than in the fall or summer ($P < 0.001$). Unweighted Unifrac pairwise PERMANOVA analysis suggested that fall and summer fecal microbiomes are highly similar, but spring is divergent ($P < 0.01$) and tightly clustered in principal coordinate analysis visualization. We conclude that substantial variation in milk and fecal microbiomes are observable over seasons representing different heat stress conditions and further hypothesize that this has a profound effect on the production and health losses described earlier. This study is the basis for a multi-institutional research initiative now underway, aiming to integrate dairy cow microbiome and transcriptome data to characterize individual responses to heat stress, providing opportunity to develop novel intervention strategies and enhance heat tolerance breeding strategies.

Key Words: heat stress, microbiome, transcriptomics

2055 Phenotypic associations between hoof lesions and type traits in Italian Holstein populations. J. Layton^{*1}, L. Benzon¹, R. Finocchiaro¹, M. Marusi¹, F. Testori², and M. Cassandro^{1,3}, ¹*Associazione Nazionale Allevatori della Razza Frisone, Bruna e Jersey Italiana (ANAFIBJ), Cremona, Italy*, ²*DVM, FA.MA. Services, Milan, Italy*, ³*Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), University of Padova, Legnaro, Italy*.

Hoof lesions are a leading cause of unwanted culling in the dairy industry, contributing to lameness, production losses, and, in severe cases, mortality. Although over 51,000 hoof trimming records have been collected to date, there is not yet enough data for a hoof health trait. However, historical classification traits may provide a proxy trait until a sufficient lesion data set can be established. This study examines the relationship between hoof lesion data and type traits in first-parity Italian dairy cows. After filtering to a single parity and merging with corresponding classification records, 6,304 binary lesion records remained from 126 herds. Preliminary analyses revealed a small but significant association between lesion presence and specific type traits. Locomotion scores showed a weak negative correlation with lesion presence (correlation = -0.1004 , $P < 0.05$) and an effect size of 0.211. Similar trends were observed for feet and leg scores, with an effect size of 0.240. However, total type scores showed no significant differences between cows with and without lesions. These findings suggest that, although classified traits can indicate lesion presence, there is not enough meaningful evidence for selection against hoof lesions with classification traits. Further research using genotypic analyses is required to determine the genetic correlations between lesions and type traits, providing a foundation for more effective breeding strategies targeting hoof health.

Key Words: hoof health, type traits

2056 Indirect genomic predictions for feed efficiency and methane emissions in Holstein cows. G. Oliveira Jr.^{*}, G. Vargas, D. Gonzales-Pena, M. Sanchez, and N. Vukasinovic, *Zoetis Genetics, Kalamazoo, MI*.

Effects of SNPs can be derived by back-solving genomic estimated breeding values (GEBVs). These predicted SNP effects enable the calculation of indirect genomic predictions (IGP) for individuals by summing the SNP effects weighted by their respective gene content. This approach is particularly useful when incorporating a large number of genotypes into a model is challenging, as well as for traits that are difficult or costly to measure, such as feed efficiency or methane emissions. The objec-

tive of this project was to predict genomic breeding values for residual feed intake (RFI; kg/d) and residual methane production (RMet; g/d) of Holstein cows using IGP. The data were collected in 5 commercial herds across the United States using the SmartFeed and GreenFeed equipment for RFI and RMet, respectively. Weekly averages of daily RFI and RMet were available for more than 4,000 Holstein cows, collected between June 2022 and August 2024. As a benchmark, a single-step (ss)GBLUP model was performed for a full data set, which included genotypes of animals with phenotype, their close relatives (spanning 5 generations), plus all available sires' genotypes, resulting in a total of around 1.5 million genotyped animals. This analysis was solved via the BLUP90IOD2 program, using as core animals a random sample of 20,000 genotyped animals for the APY method. Animals born in 2024 were set as a validation group, for which their pedigree, progeny, and own records were omitted from the data. A second ssGBLUP analysis, excluding these validation animals, was performed, and SNP effects were calculated based on the GEBV from 20,000 randomly selected genotyped animals. We obtained IGP from the SNP marker effects using PREDF90 software. The correlation between GEBV and IGP accuracies was 0.997, and the regression coefficient when regressing GEBV on IGP was 1.01 (RFI) and 1.02 (RMet). A significant reduction in computation time was observed when using IGP instead of ssBLUP GEBVs, enabling more frequent evaluations at a lower cost. These results indicate that IGP can be used as a proxy for GEBV for both RFI and RMet.

Key Words: genomics, single nucleotide polymorphism (SNP), Holstein

2235 Liver transcriptome dynamics in Holstein cows during the periparturient transition. N. Bhowmik^{*1}, M. da Silva², A. Castaneda³, A. M. Miles¹, C. Li¹, C. P. Van Tassel¹, R. L. Baldwin VI¹, E. Rico³, and G. E. Liu¹, ¹*Animal Genomics and Improvement Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service, United States Department of Agriculture, Beltsville, MD*, ²*Department of Natural Sciences, Bonn-Rhein-Sieg University of Applied Sciences, Rheinbach, Germany*, ³*Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA*.

During the peripartum phase, dairy cows undergo significant physiological, metabolic, and immunological changes that affect their health, productivity, and reproductive efficiency, as well as that of their calves. The liver plays a crucial role in helping them adapt to these challenges. This study investigated hepatic global transcriptomic changes in periparturient dairy cows in relation to energy balance, fat metabolism, and immune responses. Liver biopsies were collected from 6 multiparous Holstein cows at 21 d pre-calving (-21 d) and 7 d post-calving (7 d) for RNA sequencing. The processed RNA-seq reads were aligned to the cattle ARS-UCD2.0 genome assembly. The read counts were used for differential gene expression and co-expression network analyses. We identified 211 DEGs between -21 d and 7 d (164 up, 47 down) using a cutoff of $\log_2\text{FC} > |0.5|$ and a Benjamini-Hochberg-adjusted P -value < 0.05 , which were used for pathway enrichment. At 7 d, cows exhibited pronounced activation of genes related to fat and protein metabolism, energy balance, and cell growth, reflecting the increased energy demands of early lactation. Genes involved in tissue remodeling and lipid biosynthetic processes were downregulated, consistent with the rapid lipid mobilization required during negative energy balance. A KEGG pathway analysis indicated the activation of pathways associated with the endocrine and digestive systems, as well as fat, carbohydrate, and amino acid metabolism, and cell growth after 7 d. Conversely, the p53 signaling pathway regulating the cell cycle and DNA damage response was downregulated at 7 d, likely promoting cell survival and inhibiting

apoptosis in mammary tissue to support enhanced milk production. Co-expression network analysis further confirmed the activation of these biological processes and KEGG pathways. In conclusion, these findings provide valuable insights into the liver transcriptomic adaptations in periparturient dairy cows to meet their metabolic demands during early lactation, offering potential targets for improving health and productivity during this critical period.

Key Words: gene expression, Holstein, periparturient period

2236 Adding microbiome information to breed for reduced methane emission. A. C. Bouwman*, H. Honerlagen, R. Bonifazi, and D. Flossdorf, *Wageningen University & Research, Wageningen, the Netherlands*.

Methane is produced in the rumen of cattle by microorganisms during the fermentation of feed. Currently a great deal of attention has been paid to breed for reduced methane emission by genomic selection based on methane production measured by breath analysis. Several studies have shown that the microbiome explains part of the variation observed in methane emission. It has also been shown that part of the explained variation is due to the host genetics. The aim of our study was to identify

microbes that are associated with the methane production of cows and are also heritable, to include those in the genetic evaluation for breeding values to reduce methane emission exploiting existing genetic variation in the dairy population. Methane production measured by sniffers and rumen metagenomic data were available for 970 genotyped dairy cows from 12 farms. Using linear mixed models exploiting the cow genotypes to generate a genomic relationship matrix, we ran 2 models for each of the 577 genera with a nonzero abundance value in at least 20% of the samples: (1) with methane as phenotype and the abundance of each genus as covariable to identify rumen microbiome genera that have a significant influence on methane and are hence associated with methane emission in our cow population; and (2) with the abundance of each genus as phenotype to determine the heritability of each genus to test whether the host genetics affects the abundance of the genus. Using these 2 models we have identified a set of 31 genera that are both associated with methane and heritable, and could thus be used in selection for reduced methane emission. We are currently working on incorporating these genera set into a selection index to further improve methane breeding values.

Key Words: genetics, methane, microbiome

Dairy Foods: Dairy Products—Posters

2057 Sensory attributes that drive liking of Colby Jack sliced cheese. Y. Liu*, M. Shriner, and M. A. Drake, *North Carolina State University, Raleigh, NC.*

The global cheese market is estimated to reach \$283 billion in 2030. Colby Jack cheese is a popular American cheese. This study investigated drivers of liking and dislike in Colby Jack sliced cheeses. Duplicate lots of commercial Colby Jack sliced cheeses ($n = 9$) were evaluated cold and hot in duplicate by a highly trained sensory panel using an established lexicon. Colby Jack cheese consumers ($n = 178$) evaluated the cheeses cold in a 2-d consumer acceptance test with a partial presentation across 2 d. A separate cohort of 176 consumers evaluated the cheeses hot as an open-face grilled cheese sandwich in a 2-d consumer acceptance test with a partial presentation across 2 d. Multiple statistical analyses were conducted, including preference mapping to identify drivers of liking for consumers and consumer clusters. Colby Jack sliced cheeses differed in color, texture attributes, and milky, whey, milk fat, diacetyl, sulfur, plastic, and brothy flavors, as well as sour and salty tastes. For cold Colby Jack cheese slices, drivers of liking for all consumers were high aroma intensity, sweet taste, and a firm texture that fractured on first bite (fresh young cheese texture). Drivers of dislike were high whey flavor and sour taste. One cluster of consumers (cluster 1, $n = 132$) was distinguished by also disliking oxidized whey flavor and a texture with a high degree of breakdown and adhesiveness. Cluster 2 consumers ($n = 46$) liked diacetyl and sulfur flavors, salty taste, and more intense orange and white color. For grilled Colby Jack cheese slices, drivers of liking for all consumers were cooked/milky, milk fat, and whey flavors and a high ratio of visible orange to white color. Drivers of dislike were brothy and caramelized flavors, sweet taste, oiliness, and moisture release. Two consumer clusters were identified, which were comprised of discerning grilled cheese consumers (cluster 1, $n = 62$) with the drivers already indicated, along with salty taste and stretchability, and general consumers (cluster 2, $n = 114$) who were aligned with the drivers for all consumers. Consumers used the words “rich,” “smooth,” “buttery,” and “creamy” to describe Colby Jack cheese slices that they liked in either format.

Key Words: Colby Jack cheese, preference mapping

2058 Multiplex qPCR for simultaneous quantification of the main psychrotrophic bacteria and their enzymes in raw milk. J. Wu, L. Meng, N. Zheng, and J. Q. Wang*, *Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P.R. China.*

Psychrotrophic bacteria can thrive at chilled temperatures in milk, and can even produce extracellular heat-resistant proteolytic and lipolytic enzymes. These enzymes retain partial activity after heat treatments, contributing to protein and fat hydrolysis. Most psychrotrophic bacteria widely distributed in dairy farms, both gram-positive bacteria such as *Bacillus* and *Streptococcus*, and gram-negative bacteria such as *Acinetobacter*, *Pseudomonas*, *Stenotrophomonas*, and *Yersinia*, along with their heat-resistant hydrolytic enzymes, are of particular concern. Traditional quantification methods, such as ISO 17410-2019 and China's NY/T 1331-2007 require up to 10 d, posing inefficiencies. This study aimed to develop a multiplex qPCR assay using specific TaqMan probes for simultaneous quantification of *Acinetobacter*, *Bacillus cereus*, *Pseudomonas*, and *Stenotrophomonas maltophilia*, and their proteases/lipases in raw milk. Forty-six strains spanning 10 genera were used to validate primer/probe specificity and optimize single/multiplex qPCR conditions. The protocol comprised an initial pre-denaturation at 95°C for 3 min,

followed by 40 cycles of denaturation at 95°C for 5 s, annealing at 56°C for 15 s, and extension at 72°C for 30 s. Then, serial dilutions of 6-h bacterial cultures were prepared for DNA extraction as PCR templates, and standard curves correlating Ct values with bacterial concentrations (log cfu/mL) were established to assess assay functionality and primer-probe interactions. To assess the feasibility of the qPCR assay, spiked samples were analyzed. All tests were performed in triplicate, and negative control was included in each experiment. Validation data showed correlation coefficients >0.98 and amplification efficiency between 90% and 115%. Detection limits for the multiplex qPCR were determined to be 10^1 cfu/mL for housekeeping genes and 10^2 cfu/mL for enzyme-coding genes. Notably, the multiplex qPCR performed comparably in milk matrices and pure cultures. This method provides an assessable, time-efficient tool for simultaneous detection of psychrotrophic bacteria and their spoilage enzymes, enabling enhanced contamination monitoring and dairy quality control.

Key Words: psychrotrophic bacteria, protease and lipase, multiplex qPCR

2059 Viability of probiotics in goat milk yogurt containing plant-based prebiotics during refrigerated storage. R. Attai*,¹ M. T. Núñez de González¹, S. Woldeesenbet¹, A. Mora-Gutierrez¹, and Y. Jung², ¹*Prairie View A&M University, Prairie View, TX*, ²*Texas A&M University, College Station, TX.*

Yogurt is commonly consumed as a healthy food due to its high nutritional value and health benefits. This product has been considered a good carrier of prebiotics, probiotics, and nutrients. Supplementation of yogurt with hemp protein concentrate (HP) and carrot powder (CP) as prebiotics may give sufficient protection to probiotics in the human gut. We investigated the viability of probiotics in goat milk yogurt containing plant-based prebiotics on d 1 of production and during storage for 28 d at 4°C. The basic mix for the yogurts had 82.85% milk, 10.68% powdered milk, and 6.47% sugar. Additionally, the mix was supplemented with CP (T1), HP (T2), and a combination of CP and HP (T3). A control (T0) yogurt without plant-based ingredients was also prepared. The mix was heated at 80°C for 30 min, cooled, and then inoculated with starter culture and 2 probiotics (2% each bacterium, YF-L812, BB-12, LA-5; Chr Hansen). Then the mixes were incubated at 42°C for 5 h. The effects of the plant-based prebiotics and probiotics on the chemical composition, pH, acidity, water-holding capacity (WHC), and microbial counts of yogurts were studied for 28 d. Data were analyzed using the MIXED model procedure of SAS. The prebiotics and probiotics did not have any effect on the chemical composition of yogurts. Compared with the control, T2 (4.92 ± 0.104) and T3 (4.90 ± 0.204) exhibited higher pH in the yogurts due to the higher populations of lactobacilli. The HP-containing yogurts had significantly more *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* compared with the control, but the numbers gradually decreased ($P < 0.05$) during storage. However, the prebiotics did not affect ($P > 0.05$) the survival of *Streptococcus thermophilus* or *Bifidobacterium*, in either treatments or at storage times. Both probiotics had higher population numbers in all treatments throughout the storage period than the minimum required number ($6 \log_{10}$ cfu/g), which is beneficial for health. Future studies will focus on optimizing the sensory attributes of probiotic-containing goat milk yogurt.

Key Words: hemp protein, carrot powder, goat milk yogurt

2060 Benchmarking raw milk for traditional and novel measures of quality. M. M. Shaposhnikov*, Z. D. Wasserlauf-Pepper, R. L. Weachock, and N. H. Martin, *Cornell University, Ithaca, NY*.

The quality of raw milk has an impact on the shelf life and quality of finished products. Traditionally, raw milk quality is determined using total bacteria count (TBC) and somatic cell count (SCC), with dairy farmers often receiving economic premiums for meeting quality thresholds. Here a longitudinal study was conducted over a 15-mo period, with 100 conventional dairy farms enrolled in the study sampled 6 times per farm. The farms represented a variety of sizes, milking systems, and various other farming practices. Samples were evaluated for 24 different quality parameters, covering both traditional (e.g., TBC and SCC) and novel measures of quality (e.g., sensory defect evaluation, total leukocyte count, proportions of neutrophils, macrophages, and leukocytes). Overall, we collected 593 bulk tank raw milk samples and conducted microbial, physicochemical, and sensory analyses. The total bacteria count was determined via plating of raw milk on Standard Methods Agar (SMA), followed by incubation for 48 h at 32°C. The mean and standard deviation for TBC was 3.52 ± 0.70 log cfu/mL. The somatic cell count was determined using the Delta CombiScope. The geometric mean for SCC was 133,000 cells/mL. To determine sensory defect scores, milk samples were pasteurized at 63°C for 30 min and evaluated by a trained 3-person panel. The sensory scores ranged from 2.0 to 9.9 with a mean and standard deviation of 8.6 ± 1.4 . The most common attributes included oxidized (101 samples) and feed (23 samples). Differential somatic cell counts were determined using the QScout benchtop on-farm milk leukocyte differential analyzer. The total leukocyte counts (TLC) had a mean and standard deviation of $140,000 \pm 90,000$, and the distribution of neutrophils, lymphocytes, and macrophages were 69 ± 6 , 22 ± 5 , and 9 ± 6 , respectively. Overall, these results will establish a baseline data set for these parameters and can be used by the dairy industry to evaluate incremental improvements on raw milk quality.

Key Words: raw milk quality, microbial, sensory

2061 Production of Greek-style yogurts with different concentration methods: Impact on textural and sensory properties. E. C. Ale*, J. A. Lucey, and D. Wilbanks, *Center for Dairy Research (CDR), Madison, WI*.

Nonfat Greek yogurts are manufactured by various methods: fortification of milk (FM), cloth straining after culturing (SC), or mechanical separation after culturing (MC). We wanted to explore whether different manufacturing methods impacted the rheological properties and key sensory attributes such as chalkiness. Yogurts (9% protein) were made with skim milk using a commercial starter culture (YoFlex Mild 2.0). Samples produced via FM were fortified with MPC 85 powder, and MC samples used a GEA KNA3 separator. Two independent trials were conducted. After 2 wk of storage at 4°C, rheological properties and microstructure (Nikon A1R-SI+) were determined. An Anton Paar rheometer (MCR 301) equipped with a cup-and-bob geometry was used for viscosity testing. After mixing and adding yogurt samples to the measuring geometry, samples rested at 25°C for 15 min before an increasing shear rate (0.01 to 300 s^{-1}). Sensory attributes were determined with a trained panel at 4 wk using 15-point scales. The pH at 2 wk of storage was 4.3 for MC and SC, and 4.6 for FM samples. Rheological properties for flow curves were successfully modeled using a Herschel-Bulkley model ($R^2 \geq 0.8$). The MC treatment exhibited the lowest consistency indices (k) and highest flow indices, whereas SC had the highest k -values. Viscosity values at 50 s^{-1} were 4,270 cP for SC yogurts, 2,200 for FM, and 1,850 for MC yogurts. Micrographs showed that FM and SC samples exhibited a less dense network with large pores, whereas MC yogurts

presented a more homogeneous network with numerous, small pores. The low consistency indices, as well as the homogeneous microstructure found in MC samples, could be due to the mechanical shear applied. Chalkiness and visual gel structure were higher in all the treatments compared with non-concentrated samples ($P < 0.05$), and chalkiness was not obvious in acid whey samples derived from these yogurts. The SC yogurts presented the highest viscosity, and FM samples had the highest graininess scores. Overall, the concentration method influenced the textural and sensory attributes of nonfat Greek yogurt.

Key Words: high-protein yogurt, concentration method, textural and sensory properties

2062 Modeling the melting behavior of high-protein ice creams. N. Nkansah, K. Pizarro, J. Olatunde, and S. Martinez-Monteagudo*, *New Mexico State University, Las Cruces, NM*.

High-protein ice cream consists of a protein increment of about 4- to 6-fold compared with regular ice cream. Industrially, these ice creams are formulated with blends of proteins to achieve the desirable concentration of protein (6%–8%). Such concentration of protein alters the flow behavior of the mix and therefore the resulting quality of the ice cream. This work aims to model the melting curves (torque vs. temperature) of ice creams formulated with 4 different sources to achieve 7.5% of protein, including WPC-80, MPC-85, MCC, and calcium caseinate. The melting behavior was evaluated through oscillatory thermorheometry within the temperature range of -20 to 10°C . All samples exhibited 3 distinct zones: zone I (-20 to -10°C), zone II (-10 to 0°C), and zone III (0 to 10°C). The tangent loss factor ($\tan \delta = G''/G'$) was also used to evaluate the melting curve, where the temperature corresponding to the highest value of $\tan \delta$ varied from -4.4 to 9.8°C , depending on the protein source. For instance, the highest value of $\tan \delta$ (0.61 ± 0.02) was found at $-5.4 \pm 0.15^\circ\text{C}$ when WPC-80 was added. On the contrary, the highest $\tan \delta$ (0.59 ± 0.03) was found at $-9.8 \pm 0.18^\circ\text{C}$ when calcium caseinate was used as a protein source. The melting curves were modeled using 4 equations: a logistic model, the Gompertz model, the Richard model, and the Hill model. The experimental melting curves were adequately represented by the logistic model, judging by several criteria ($R^2 = 0.999$, adjusted $R^2 = 0.999$, and Akaike probability = 0.294). The analysis of the oscillatory curves can be used to classify the melting behavior of high-protein ice creams.

Key Words: ice cream, modeling, melting

2063 Probiotic-fortified camel milk whey powder. M. Mbye* and A. Ali, *Khalifa University, Abu Dhabi, United Arab Emirates*.

Camel milk differs from cow milk in several key aspects, particularly composition and functionality. It has lower total protein, with significantly less β -lactoglobulin and α_{S1} -casein. Camel milk contains more lactoferrin and immunoglobulins, contributing to its antimicrobial and immune-boosting properties. Camel milk whey powder holds promise for various food industry applications. The absence of β -lactoglobulin in camel whey, a significant allergen that accounts for 50% of cow milk whey protein, makes camel-derived whey protein a safer alternative for individuals with dairy allergies. The study's objectives are to evaluate the effect of spray drying on the survival of whey powder's probiotic and techno-functional properties. The study employed 3 batches of camel whey protein fortified with the probiotic *Bifidobacterium* with an initial fortification of 10^{10} cfu/mL and then spray-dried at temperatures of 160°C , 170°C , and 180°C , with freeze-drying as a control. All analyses were conducted in triplicate ($n = 3$). Statistical

analyses were performed using ANOVA to determine significant differences among treatments, with Duncan's multiple range test applied for post-hoc comparisons. Probiotic viability was measured using plate counting, revealing a survival rate of 10^8 cfu/g in the powder at 160°C. The structural analysis, using Fourier-transform infrared spectroscopy and X-ray diffraction, indicated no significant differences in functional group profiles between spray-dried samples and the control. However, the control sample retained some crystallinity, meaning that a portion of the sample's structure remained crystalline rather than being completely amorphous. This suggests that not all material fully transitioned to a non-crystalline (amorphous) form during processing. The crystallinity was measured with X-ray diffraction, which identifies crystalline regions by detecting characteristic diffraction, whereas spray-dried samples showed minimal crystallinity. The powders' surface morphology, particle size, and surface charge were analyzed through scanning electron microscopy and a Zetasizer. Particle size refers to the dimension (diameter) of individual particles in a sample, measured in nanometers (nm). The particle size distribution among the samples at different temperatures is as follows: 160°C: 400.0 ± 15 μ m; 170°C: 352.1 ± 6.4 μ m; and 180°C: 282.7 ± 10 μ m. These values indicate that particle size decreases significantly as drying temperature increases. Color was measured with a Chroma meter. The spray-dried samples were whiter than the control, with higher L* values of 91.57 ± 0.01 ($P < 0.05$). Additionally, the sample spray-dried at 170°C achieved the highest protein solubility (98%), compared with 90.7% in the sample dried at 160°C. The highest emulsifying activity index, 143.75 m²/g, was also noted in the 170°C spray-dried sample. In conclusion, camel milk whey powder spray-dried under optimal conditions displayed favorable physico-chemical, technological, and functional qualities, making it a viable alternative to cow milk whey powder.

Key Words: camel milk whey, probiotic, spray dried.

2064 Unraveling flavor dynamics in the production of milk protein concentrates. V. Tahiri*, J. K. Amamcharla, and F. F. G. Dias, *Department of Food Science and Nutrition, University of Minnesota–Twin Cities, Saint Paul, MN.*

The demand for low-acid high-protein dairy beverages (HPDB) is growing, emphasizing the importance of milk protein concentrate (MPC) as a key ingredient. To meet consumer expectations, MPC must preserve its “milky” flavor while minimizing off-flavors. However, the milky flavor diminishes with higher-protein MPC, leading to reduced performance in food systems. This study investigates how ultrafiltration (UF) and diafiltration (DF) influence retention of milky flavor in MPC. Pasteurized skim milk was processed using a 10-kDa polyethylene sulfone (PES) membrane with 100% DF to produce MPC (87% protein). Volatile compounds in the starting skim milk, retentate, and permeate were analyzed using headspace solid-phase microextraction (SPME) gas chromatography-mass spectrometry (GC-MS) in triplicate. More than 20 volatile compounds were identified in the skim milk (ketones [32%], alkanes [26%], organic acids, aldehydes, alcohols, and esters). Using one-way ANOVA with Tukey comparison of means, the UF/DF process resulted in a significant ($P < 0.05$) reduction of volatile compounds in the retentate (6 compounds, alcohols [33%], aldehydes [17%], and ketones [17%]), whereas 27 volatile compounds were found in permeate (alcohols [30%] and ketones [26%]). This finding shows a challenge in retaining flavor compounds from skim milk to retentate during UF/DF. Hexanal (green, grassy) and nonanal (citrus, soapy) were oxidation markers and present in all samples. Retentate contained significantly higher concentrations of hexanal ($P < 0.05$) and nonanal ($P < 0.05$) than permeate. Hexanal, 2-ethyl- (fruity, sweet) was only found in the

retentate and not in the skim milk. Compounds such as butanoic acid (milky, sweaty), pentanal (green, fatty), and heptanoic acid (sweaty) were present in the skim milk and permeate but absent in the retentate, highlighting flavor retention challenges. These findings reveal significant flavor migration and loss during UF/DF processing and underscore the need to optimize process conditions to retain desirable flavors in MPC. This study establishes a foundation for future research to enhance MPC flavor retention for HPDB applications

Key Words: ultrafiltration, volatiles, flavor, milk protein concentrate (MPC)

2065 Impact of proportion of pasture in the cow diet and seasonality on the milk metabolome as determined by ¹H-NMR. P. Rojas-Gómez*, R. Pariyani¹, L. Bateman^{2,3}, D. Lynch², M. Timlin⁴, M. Dineen⁵, N. A. McCarthy⁴, A. Brodkorb⁴, A. R. Maguire^{2,3}, M. O'Donovan⁵, D. Hennessy⁶, T. B. Murphy⁷, J. A. O'Mahony¹, and T. F. O'Callaghan¹, ¹*School of Food and Nutritional Sciences, University College Cork, Cork, Ireland*, ²*School of Chemistry, Analytical and Biological Chemistry Research Facility, University College Cork, Cork, Ireland*, ³*School of Pharmacy, University College Cork, Cork, Ireland*, ⁴*Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland*, ⁵*Teagasc Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland*, ⁶*School of Biological, Earth and Environmental Sciences, University College Cork, Distillery Fields, North Mall, Cork, Ireland*, ⁷*School of Mathematics and Statistics, University College Dublin, Dublin, Ireland.*

This research explores how the proportion of pasture (high, medium, or no pasture) influences the milk metabolome throughout lactation in a seasonal spring-calving dairy system, using proton nuclear magnetic resonance (¹H-NMR) spectroscopy. A total of 54 spring-calving cows were assigned to the 3 dietary regimens for the duration of lactation. Weekly bulk milk samples ($n = 111$) were collected from each herd over 37 weeks (March–November), and 43 metabolites were quantified from a variety of chemical classes, including amino acids and nitrogenous compounds, carbohydrates, volatile fatty acids, organic acids, and alcohols. Significant dietary and seasonal effects on the milk metabolome were identified, revealing distinct metabolome profiles associated with varying pasture allowance and seasonal shifts. Receiver operating characteristic (ROC) analysis highlighted potential of metabolites as biomarkers for distinguishing between pasture and non-pasture-based feeding systems: for example, hippurate (associated with pasture-containing diets) and propionate, lactate, and isobutyrate (associated with no-pasture diet). Partial least squares discriminant analysis (PLS-DA) identified dimethyl sulfone as a key metabolite linked to high-pasture diets. These findings emphasize the complexity and variability of the milk metabolome as influenced by feeding strategies and seasonal changes. The ¹H-NMR analysis demonstrated significant potential as a technique for identifying and quantifying milk metabolites, providing deeper insights into milk composition and serving as a valuable reference data set for future research. As demand for “grass-fed” dairy products grows, metabolomic analysis could enhance traceability and authentication of milk composition throughout the year, supporting the development of novel tools for monitoring and improving the dairy supply chain.

Key Words: milk metabolome, pasture, cow diet

2066 Developing a digital infrastructure for implementing milk shelf-life prediction model. C. Qian*, A. Trmcic, N. H. Martin, and M. Wiedmann, *Cornell University, Ithaca, NY.*

Fluid milk accounts for a significant proportion of food waste in the United States. Limited adoption of digital tools hampers waste reduction, highlighting the urgent need for technological advancements. We aimed to develop and deploy a standardized digital infrastructure in a selected dairy supply chain to optimize milk spoilage prediction. We incorporated real-time data into a previously validated Monte Carlo simulation model that predicts milk spoilage due to post-pasteurization contamination and psychrotolerant sporeformers to assess the model utility in industry settings. Milk volume and temperature data were extracted from the automated data monitoring system at a dairy processor across various processing stages. We assumed a half-day transportation period from processing to retail and a 20% post-pasteurization contamination frequency, consistent with estimates for dairy plants with high-quality milk based on longitudinal data from the Voluntary Shelf-Life Program at Cornell University. We simulated 100 lots of 100 milk containers each under 3 scenarios, which were storage in the product cooler for 1 d, 7 d, and 17 d before retail delivery, with the latter 2 representing delayed transportation due to reduced demand. Simulation results showed that bottled milk reached consumers by d 5, 11, and 21 post-production. The median and 95% quantiles for the percentage of milk exceeding the sensory defect threshold (6 log bacterial concentration) on these respective days were 0% [0%–2%], 6% [3%–9%], and 18% [14%–21%], which were significantly different based on Kruskal-Wallis test. Defining shelf life as the point when 25% of containers exceed this threshold, the estimated remaining days for consumption after retail purchase were 22, 17, and 9 d for the 3 scenarios, suggesting that holding milk at the plant longer might not proportionally reduce the shelf life. These findings highlight the potential of digital frameworks to support data-driven decision-making in dairy processing, particularly in shelf-life estimation. This approach serves as a model for the digitalization of dairy supply chain management.

Key Words: Monte Carlo simulation, milk spoilage, digitalization

2067 Rheological and physical characterization of commercial dry sweet whey powders. A. Kapoor^{*1}, J. A. Lucey^{1,2}, and D. Wilbanks¹, ¹Center for Dairy Research, University of Wisconsin–Madison, Madison, WI, ²Department of Food Science, University of Wisconsin–Madison, Madison, WI.

Dry sweet whey (DSW), a cheese co-product, is widely used in dairy and food products but faces challenges due to caking and poor flowability. This study hypothesizes that time consolidation of high-lactose powders (during storage) is influenced by their flow and physical properties. The objectives were to assess DSW flowability, surface characteristics, and structural properties. Five commercial DSW samples (DSW-1 to DSW-5) were collected within 1 week of manufacture from different dairy processors, stored at room temperature, and tested within 90 d of manufacture. Laser diffraction analysis indicated particle sizes (D_{4,3}) ranging from 44 to 121 μm , with DSW-2 having the smallest size (44 μm) and highest tapped bulk density (0.87 g/mL, 625 taps). The specific surface area, measured by the Brunauer-Emmett-Teller method, ranged from 0.12 to 0.3 m²/g, indicating variations in particle surface features that influence flowability and cohesion. Differential scanning calorimetry (DSC) identified glass transition (T_g) peaks between 36 and 53°C, followed by an endothermic peak at 137–149°C, likely due to α -lactose monohydrate. Rheological testing using a shear cell assessed flow function coefficients (ffc) of powders through Mohr circle analysis. Powders with smaller particle sizes and larger surface areas, particularly DSW-1, exhibited higher cohesion and stick-slip behavior, indicating poor flowability. Trends suggested that powders with smaller particle

sizes and larger surface areas exhibited higher yield strength and cohesion. These findings suggest that composition, especially lactose content, affects the powder's rheological properties and flowability. The study explores the interplay between physical (particle size, surface area, T_g) and rheological properties (cohesion, yield strength, ffc, stick-slip behavior), providing insights into optimizing DSW powder flowability and processing for improved quality and shelf stability.

Key Words: dry sweet whey powder, flowability, caking

2068 The compositional and rheological properties of milk protein concentrate and micellar casein concentrate retentates with 62%, 74%, and 85% total protein to total solids ratios. S. Chauhan^{*}, S. Ellekuttige, M. A. Rahman, K. Sartell, S. Beckman, and P. Salunke, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Milk protein concentrates (MPC) and micellar casein concentrates (MCC) are high-protein dairy ingredients obtained through ultrafiltration (UF) and microfiltration (MF), respectively. Their composition and processing influence total protein to total solids (TP/TS) ratios, soluble constituents, and functional properties. This study examines the compositional and rheological properties of MPC and MCC retentates and permeates at TP/TS ratios of 62%, 74%, and 85%. Pasteurized skim milk (10,400 kg) was divided into 2 equal lots: one processed using UF (10 kDa) to produce MPC and the other using MF (0.2 μm) to produce MCC. Both processes were conducted at 11°C with diafiltration and recirculation to achieve higher TP/TS concentration. Retentates and permeates were analyzed for composition, particle size, zeta potential, viscosity, and viscoelastic properties (storage modulus, G', and loss modulus, G'') at 20°C. The MPC retentates had higher total solids (22.1% in MPC85) than MCC (16.0% in MCC85) and greater ash content. Particle size (~0.31–0.33 nm) remained stable, and zeta potential was consistently negative, indicating colloidal stability. Viscosity increased with TP/TS ratio, with MPC exhibiting higher viscosity than MCC. Both showed shear-thinning behavior. Viscoelastic measurements showed that MPC retentates had higher G' and G'' than MCC retentates across all TP/TS ratios and increased with the increase in TP/TS ratio, with the highest values recorded in MPC85. Permeates, on the other hand, showed no significant ($P < 0.05$) differences in viscosity or viscoelastic properties between MPC and MCC, consistently exhibiting low viscosity (~2 mPa·s) and weak elastic and viscous behavior. In conclusion, MPC and MCC retentates had distinct properties, with soluble constituent removal significantly affecting rheological properties. These findings aid in optimizing MPC and MCC for future dairy foods applications.

Key Words: milk protein concentrate (MPC), micellar casein concentrate (MCC), rheological properties

2069 Monitoring the quality attributes of shelf-stable milk products under various storage conditions. L. Li^{*1}, L. L. Bauer², R. N. Dilger², and Y.-C. Wang¹, ¹Department of Food Science and Human Nutrition, University of Illinois at Urbana–Champaign, Urbana, IL, ²Department of Animal Sciences, University of Illinois at Urbana–Champaign, Urbana, IL.

The processing and packaging of shelf-stable milk enable it to be safely stored at room temperature for an extended period. However, the heat treatment used in this product's production can cause significant chemical and physical changes, affecting product quality. One of the key mechanisms involved is the Maillard reaction, which can lead to

browning, off-flavor formation, and nutrient degradation. Additionally, the extent of such changes can be influenced by storage conditions, potentially impacting the shelf lives of these milk products. Therefore, in this study, we investigated 2 key quality attributes of commercially available shelf-stable milk that had been stored in our laboratory at 3 temperatures—22°C, 37°C, and 50°C—over 25 d. To ensure consistency, all samples were off-the-shelf items from the same lot, directly shipped to us from the vendor. The 2 attributes we measured were furosine content, an early stage Maillard-reaction marker, and color change, a key influence on consumer acceptability. Furosine was quantified following acid hydrolysis using reversed-phase high-performance liquid chromatography (RP-HPLC), and color changes were assessed in terms of L*, a*, and b* values using a Chroma Meter, both with 2 independent samples. No marked changes in b* values were found in milk samples stored at 22°C between d 0 and 25. However, browning—indicated by b* values—was observed at the 2 higher storage temperatures, and such values increased significantly ($P < 0.05$) as storage temperature increased. While the furosine levels of milk stored at 22°C and 37°C increased over the 25-d storage period, the samples stored at 50°C initially showed an increase followed by a decline. As well as providing insights into how storage conditions impact the quality of shelf-stable milk, these findings could aid the development of intelligent packaging systems or other indicators that provide real-time product-quality information, enabling consumers and other stakeholders to make informed decisions on milk retention, consumption, or disposal, thereby reducing food waste and ensuring optimal quality at the time of consumption.

Key Words: shelf-stable milk, furosine, browning

2070 Toward the rheological fingerprint of protein-enriched Greek-style yogurts. A. G. Soler Sanchez*, J. I. Agbawodike, M. A. Ur Rehman Alvi, L. E. Sabillon Galeas, and S. I. Martinez Montegudo, *New Mexico State University, Las Cruces, NM*.

Yogurts fortified with protein concentrates are frequently used as a primary ingredient in beverages, frozen desserts, and novel products, often undergoing large deformations during processing. The present study examined the linear and nonlinear rheological behavior of protein-enriched Greek-style yogurt (15% total solids) formulated with varying whey protein to casein ratios (13/87, 20/80, and 65/35) with the incorporation of micellar casein concentrate (MCC85), milk protein concentrate (MPC80), and whey protein concentrate (WPC80). The yogurt samples were prepared in triplicates through skim milk fortification (43–49°C, 30 min), heat treatment (90°C, 10 min), temperature reduction to 43°C, inoculation (0.03% wt/wt), fermentation (43°C, 5–6 h, final pH 4.6 ± 0.1), and storage at 5°C for 21 d. Compositional analysis presented crude protein contents of $29.40 \pm 0.8\%$, $26.69 \pm 0.5\%$, and $27.13 \pm 0.6\%$ for 13/87, 20/80, and 65/35, respectively. Small amplitude oscillatory shear tests demonstrated that the 13/87 ratio exhibited the longest linear viscoelastic region ($\gamma_c = 48.95\%$) compared with 20/80 (30.46%) and 65/35 (35.83%). Frequency sweeps indicated weak gel-like behavior ($G' > G''$) across all samples. Large amplitude oscillatory shear analysis indicated moderate nonlinear elasticity ($e_3/e_1 > 0$), with elastic Chebyshev coefficient ratios of 0.45, 0.60, and 0.50 for 13/87, 20/80, and 65/35 ratios, respectively. The 20/80 ratio displayed the highest degree of intracycle strain stiffening ($S = 0.69$), indicating improved recovery from large deformations. Lissajous-Bowditch curves showed strain thinning behavior for all formulations. Scanning electron microscopy images displayed that the 65/35 yogurt had a compact protein network with small pores, which corresponded to the lowest ($P < 0.05$) syneresis ($32.51 \pm 2.54\%$) and hardness (2.01 ± 0.08 N) after 21 d of cold storage. The outcomes of this investigation suggest that protein composition is a

determining factor in the structural integrity and functional performance of yogurt, influencing its processing and storage stability.

Key Words: protein ratio, large amplitude oscillatory shear analysis (LAOS), microscopy, yogurt

2071 Evaluation of retorted nutritional beverages: Impact of calcium carbonates and pH on viscosity, emulsion stability, and mechanism. D. C. Strozier* and P. A. Haselberger, *Abbott Nutrition, Columbus, OH*.

Achieving optimal viscosity and stable emulsion properties in fiber-enriched nutritional beverages is challenging. The fiber source is a proprietary blend of soluble and insoluble fibers. This study examines the effects of pH and calcium carbonate on beverages containing caseinate and soy protein after retort sterilization (128°C for 3 min). Calcium carbonate A (CC-A) has higher soluble calcium, and calcium carbonate B (CC-B) has lower soluble calcium. Formulations were batched with these carbonates: Batch 1 (CC-A) and Batch 2 (CC-B), with pH adjusted to 6.5–7.5 before sterilization. Post-sterilization samples were tested for pH, viscosity, protein aggregation, protein degradation, soluble protein, particle size, and emulsion stability. Half the samples were also tested for soluble calcium, the ratio of soluble to whole calcium, and qualitative insoluble fiber analysis. In analysis, *t*-tests revealed significant differences ($P < 0.05$) between batches. Batch 1 had higher viscosity, lower particle size, higher soluble protein, and lower emulsion stability. For Batch 1, increasing pH from 6.5 to 7.5 resulted in viscosity ranging from 58 to 124 cP, whereas Batch 2 showed a narrower range (56 to 79 cP). Higher pH (7.3–7.5) in Batch 1 led to more insoluble fiber precipitation. The pH had no effect on protein aggregation. The lower soluble calcium of CC-B interacts less with casein micelles and soy proteins during heat treatment, resulting in a narrow viscosity range. The higher soluble calcium of CC-A interacts more, causing greater gelation and thickening. At higher pH, Batch 1 showed increased insoluble fiber precipitation.

Key Words: dairy nutritional beverage, viscosity, emulsion

2072 Retrospective evaluation of raw milk from Rondônia, Western Amazonia. S. V. Pascoal¹, E. M. Reis*¹, F. L. Dantas¹, C. T. Messias¹, A. B. Siqueira², L. F. Moraes¹, L. C. Mota¹, and M. E. Lima¹, ¹*Federal University of Acre, Rio Branco, Acre, Brazil*, ²*Federal University of Roraima, Boa Vista, Roraima, Brazil*.

This study evaluated the quality of raw milk in the central region of Rondônia in 2021, considering the parameters established by Normative Instruction No. 76/2018 of the Ministry of Agriculture, Livestock, and Supply (MAPA–Brazil). A total of 140 samples were analyzed, assessing physicochemical aspects (nonfat dry extract, fat, lactose, protein, and total solids) and microbiological aspects (total bacterial count [TBC] and somatic cell count [SCC]). The samples were placed in 40-mL vials with Bronopol preservative. The analyses were performed by mid-infrared (MID) absorption and infrared spectroscopy, Fourier-transform spectroscopy (International Dairy Federation), according to the official methodology of Normative Instruction No. 77 (MAPA–Brazil). The results showed that SCC remained within the legal limit ($<500,000$ cells/mL), whereas TBC exceeded the permitted threshold ($>300,000$ cfu/mL) in 3 quarters, affecting 39 producers. The fat content in February (2.65 g/100 g), and April (2.78 g/100 g), below the minimum required (3.0 g/100 g). Variations in fat content can be attributed to pasture quality and lack of adequate supplementation. The peaks in TBC are associated with inadequate milking and storage hygiene. The analyses covered can help dairy farmers detect health, nutrition, or herd management

issues more quickly, and implement changes to improve milk quality and characterization in the industry. It is concluded that the raw milk analyzed between January and December presented nonconformities in fat content and fluctuations in TBC, indicating the need for improvements in management, feeding, and hygiene practices to ensure product quality and safety.

Key Words: dairy production, milk quality, sanitary inspection

2073 Analysis of the composition and physical properties of commercial high-protein chocolate milks. R. Choriego*, B. Patel, and R. Jimenez-Flores, *The Ohio State University, Columbus, OH.*

Chocolate milk is a highly liked dairy product. Recently, new milks have been launched to market with enhanced functionality for consumers. However, there has been little public research on these products as food systems. The objective of this study is to compare the composition and physical properties of commercial chocolate milks with different protein content to identify their distinctive characteristics. Eight chocolate milks commercially available in the United States were used for this study. The total solids, protein, and ash were directly analyzed, with SDS-PAGE used to identify the proteins' molecular weights. The physical properties evaluated were instability index, thermal properties, and viscosity. Instability index was determined using a LUMiSizer dispersion analyzer at 4 and 25°C. Viscosity was evaluated in a rheometer performing a temperature ramp from 4 to 85°C at a shear rate of 10·s⁻¹. Thermal properties were analyzed using 2 temperature cycles from -40 to 95°C using a differential scanning calorimeter to determine onset and peak temperatures, enthalpy, and water frozen as ice. All samples matched their nutritional labels for total protein. The total solids of all samples ranged from 12.0% to 13.3% despite their protein content, confirming the removal of components as lactose to account for protein increase. There were significant differences in the ash content between samples, but all values were in the range of 0.7% to 1.3%. The instability index kept the same trend at both temperatures, but it was not related to the protein in the samples. The use of cellulose-based stabilizer had a significant effect on physical properties of products, especially a higher viscosity. The most significant analytical values indicate that the equilibrium between protein content and calcium are related to the different stabilizing systems used in the different formulations. Key characteristics of these products beyond their protein content were determined, showing their variability and potential factors influencing consumers' perception and preferences toward them.

Key Words: novel dairy drink, high-protein beverage

2074 Screening of exopolysaccharide-producing lactic acid bacteria from Mexican cheeses and cocoa bean fermentation for use as adjunct culture in kefir fermentation. H. G. Gutierrez-Ríos*, M. O. Ramírez-Sucre², M. L. Suárez-Quiroz¹, Z. J. Hernández-Estrada¹, C. Y. Figueroa-Hernández¹, O. González-Ríos¹, and S. T. Del Moral Ventura¹, ¹*Instituto Tecnológico de Veracruz-TECMN, Veracruz, Veracruz, Mexico*, ²*Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco (CIATEJ), Mérida, Yucatán, Mexico.*

Exopolysaccharides (EPS) produced by lactic acid bacteria (LAB) are key compounds in dairy products, such as kefir, as they improve texture, viscosity, stability, and sensory properties. This study aimed to screen EPS-producing LAB strains isolated from different sources for potential use as adjunct cultures in kefir production. The LAB strains used were isolated from Mexican cheeses, whey cheese, and cocoa bean fermentation. The EPS-producing strains were selected on MRS agar

with 10% sucrose. A 48-h kinetic study at 30°C in MRS broth (10% sucrose) assessed viable cell counts, pH, total titratable acidity (TTA), EPS production, and viscosity. Milk acidification was evaluated by monitoring pH, TTA, and viable cell counts in whole milk supplemented with 2% glucose and 1% yeast extract. Among 115 LAB isolates, only 4 strains exhibited aropy wire-drawing phenotype indicative of EPS production: one from cocoa (B4) and 3 from cheese (QJ0624, QJ1324, SP0724). Strain QJ0624 had the highest EPS yield (16.68 ± 0.73 mg/mL), followed by QJ1324 (14.63 ± 0.69 mg/mL), B4 (12.00 ± 0.34 mg/mL), and SP0724 (8.67 ± 0.06 mg/mL). Similarly, strain QJ0624 showed the highest viscosity (16.4 ± 0.3 mPa·s), whereas B4 (8.8 ± 0.3 mPa·s) had moderate viscosity, and QJ1324/SP0724 exhibited lower values (2.9 ± 2.5 mPa·s). Differences between EPS yield and viscosity suggest variations in polymer composition, molecular weight, and strain-specific interactions with the medium. Acidification kinetics showed that, in 12 h, B4 had the highest acidification rate (pH 4.5, TTA 0.81%), whereas cheese-derived strains had lower TTA (0.67%). These results suggest that the screened LAB strains effectively ferment milk, promoting the acidic conditions necessary for kefir fermentation enhancement. In conclusion, the selection of strains QJ0624 and B4 as potential adjunct cultures is based on their high EPS production and viscosity, which are critical for improving kefir's texture and stability. Their application in kefir fermentation could contribute to developing functional products with enhanced sensory properties.

Key Words: kefir, exopolysaccharide (EPS), adjunct culture

2075 Exploring the potential of dry yogurt acid whey as a clean-label, value-added ingredient for enhanced sensory attributes in pancake and scone dry-mix formulations. D. Heaney*, A. Garg, and O. Padilla-Zakour, *Cornell University, Ithaca, NY.*

Dry yogurt acid whey (DYAW) is the powder produced after concentrating and freeze-drying yogurt acid whey (YAW), a by-product of Greek yogurt. Recent research highlights YAW as an upcycled, value-added ingredient in products such as ranch dressing, sports beverages, and pizza crust, given its high mineral, lactose, and natural acid contents. We explored the use of DYAW in 2 clean-label, baked dry-mix formulations (just add water and fat) for pancakes and scones. Formulations were created using 12% dry basis weight (dwb) DYAW in the pancake mix and 5.24% dwb in the scones. For control samples, the same percentage of buttermilk powder was used, as the ingredient gives taste and texture profiles similar to those of DYAW. The sensory attributes of the prepared baked goods and their overall consumer acceptability were analyzed (n = 112 for pancakes; n = 106 for scones). Data on overall liking, purchase intent, flavor and texture profiles, and consumer preferences were reported. Treatments were prepared in triplicate and statistical analyses performed at 95% confidence level. Addition of DYAW resulted in slightly greater sweetness than the control pancake, which could be due to the higher amounts of lactose and galactose in DYAW. The acidic nature of DYAW helped in improving the leavening of the batter, which resulted in improved chewiness, fluffiness, and moistness attributes of the prepared pancake compared with the control sample. Overall liking and flavor liking of DYAW scones were similar to the control (P > 0.05), and there was no difference when consumers were asked to rank the samples. There was a significant perceived difference in crumbliness and moistness of the samples. However, purchase intent of DYAW scones did not change, suggesting consumers have different perceptions of an ideal scone, which can include DYAW. Our results indicate the potential of DYAW as a sustainable, value-added ingredient for improved attributes such as sweetness and fluffiness in baked goods. Using DYAW made a cleaner label product by removing 2 chemical

leavening agents, SAPP-40 and MCP. Future studies will investigate textural changes over time in both dough and final goods using texture profile analysis, as well as shelf-stability.

Key Words: acid whey, baked goods, sensory

2076 Effect of casein-derived Maillard reaction products on the intestinal microbiome using the Simulator of Human Intestinal Microbial Ecosystem (SHIME), a multicompartment dynamic simulator of the human gut. R. Ward*¹, M. Ben², A. Polo², R. Di Cagno², and M. Gobetti², ¹*Nutrition, Dietetics and Food Sciences, Utah State University, Logan, UT*, ²*Faculty of Agriculture, Environmental and Food Sciences, Free University of Bolzano, Bolzano, Italy*.

Heat processing of food promotes 3 chemical changes that affect the human gut microbiome as well as metabolic health: the Maillard reaction, lipid oxidation, and vitamin destruction. Rodent studies show that baking rodent diet pellets promotes insulin resistance, when compared with unbaked pellets, and the effect is often attributed to Maillard reaction products (MRPs). When we provided casein-derived MRPs to mice with normal vitamin levels and unoxidized fats in their diets, we found a dose-dependent increase in insulin sensitivity and significant changes in the gut microbiome. The objective of this project was to use the Simulator of Human Intestinal Microbial Ecosystem (SHIME), a dynamic in vitro gut model to determine specifically how casein-derived

MRPs affect the composition and metabolic activity of the microbiome compared with unheated casein. The MRPs studied were produced via heating using a mixture of bovine casein, sucrose, glucose, and fructose, reflecting a similar mixture previously used in rodent diets. The SHIME bioreactor was inoculated with a verified Mediterranean-diet-type human microbiome and allowed to stabilize for 2 weeks. Subsequently, in a parallel-line design, the SHIME was fed every 8 h with 140 mL of feed (SHIME medium + 16 g of protein + 21 g of sugars) of either the control (unheated casein sugars) or the MRPs (heated casein sugars) plus 60 mL of pancreatic juice. Samples were taken at baseline, 3 d and 7 d of treatment, and after a 1-wk washout. The composition of the microbiome was phylotyped using 16S RNA sequencing, and the metabolic activity was measured via medium short-chain fatty acid (SCFA) concentration. The results indicated a significant effect of the treatment on α diversity of the microbiome in the proximal, but not the distal colon, and α diversity was significantly different in both colon tracts as a function of time. For β diversity, there was an interesting differentiation of the time course of the treatment as evidenced by principal components analysis plots. Interestingly, inclusion of MRPs into the SHIME treatment line was associated with a significant increase in propionate in the proximal colon and butyrate in the distal colon, which is similar to results found in previous studies in mice. Collectively, these results suggest that casein-derived MRPs may have beneficial effects on the microbiome by serving as an energy source for specific microbial communities.

ADSA-GSD Competition: Dairy Foods Poster Semifinalists

2077 Defining creaminess in ice cream. B. Adams*, M. E. Watson, and M. A. Drake, *North Carolina State University, Raleigh, NC.*

Creaminess is an attribute associated with consumer product acceptance. Previous studies have demonstrated that components (extrinsic and intrinsic) that define creaminess are product-specific. The objective of this study was to characterize the consumer perception of creaminess in ice cream. Ice cream consumers were recruited for four 2-h focus groups ($n = 30$) and an online survey ($n = 433$). The focus groups addressed consumer definitions of creaminess in ice cream as well as intrinsic and extrinsic cues consumers use to identify creamy ice cream. Qualitative beliefs from focus groups were compiled to generate a creaminess definition map; these findings were used to develop survey questions. The online survey used check-all-that-apply (CATA) questions, sliding scales, a build-your-own (BYO) exercise, agree/disagree statements, a maximum difference (MXD) exercise, and an ingredients list activity to further explore and quantify results obtained in the focus groups. Univariate and multivariate analyses were applied to survey data. Consumers in focus groups confirmed creaminess as a desirable ice cream attribute and identified texture, temperature, and flavor components as important to creamy ice cream. These findings were confirmed by survey participants. Texture and density/thickness were identified as most important to the creaminess of ice cream by 74% and 61% of survey participants, respectively, followed by ingredients, scoopability, flavor, and mouthcoating. Vanilla was the creamiest ice cream flavor ($P < 0.05$). In the BYO exercise, the creamiest vanilla ice cream was smooth, with a high fat content, melted slowly, coated the mouth, was easy to scoop, dense/thick, and had a strong vanilla flavor. Ice cream ingredient decks with fewer ingredients were perceived as creamier than longer ingredient decks ($P < 0.05$). These findings provide a platform to explore optimum parameters for creaminess in ice cream.

Key Words: ice cream, creaminess

2078 What does “organic” mean to today’s milk consumer? A. R. Best* and M. A. Drake, *North Carolina State University, Raleigh, NC.*

Organic milk remains a popular choice for fluid milk consumers and is a value-added product with a price premium. Previous work has demonstrated that the belief that organic milk is healthier than conventional milk drives the purchase of organic milk. There has not been a recent study focused on consumer perception of organic fluid milk. The current study evaluated the purchase drivers for organic and conventional milk and consumer beliefs behind those drivers. An online survey ($n = 510$ milk consumers) was launched with consumers at East and West Coast locations in the United States. Maximum difference (MXD) scaling, check-all-that-apply, and sliding-scale questions were used to assess consumer beliefs and fluid milk messaging. Adaptive choice-based conjoint (ACBC) analysis was applied to determine the importance of specific fluid milk attributes (brand, flavor, type, label claim, free-from claims) on milk purchase. Analysis via ANOVA with means separation was applied to sliding-scale values. Hierarchical Bayesian (HB) estimation was applied to MXD and ACBC data to determine utility scores. Segmentation of MXD utility scores was performed using latent class analysis. The ideal fluid milk for all consumers was 2% fat milk in a half-gallon container with the following claims: USDA organic seal, hormone-free, and locally farmed. Conventional consumers ($n = 299$) stated cost as the main reason for not purchasing organic milk and

assigned higher value to cost and container size than organic consumers ($P < 0.05$). Likewise, organic consumers ($n = 211$) assigned higher value to pasture/grass-fed and antibiotic- and hormone-free label claims, and ethical farm/animal welfare practices than conventional consumers ($P < 0.05$). Both milk consumer groups believed organic milk was more ethical than conventional milk ($P < 0.05$), but conventional consumers assigned the same ethicality score to fluid milk with an all-natural claim ($P > 0.05$). Organic milk consumers were segmented but overall identified organic milk as healthier, higher-quality, and more ethical than conventional milk ($P < 0.05$). These results provide the fluid milk industry with actionable information for strategic messaging and education for fluid milk consumers.

Key Words: fluid milk, organic, consumer

2079 Child acceptance of school lunch milk with different heat treatments. E. Warner*¹, D. Shah³, K. Bhanduriya³, D. M. Barbano², and M. A. Drake¹, ¹*North Carolina State University, Raleigh, NC*, ²*Cornell University, Ithaca, NY*, ³*Byrne Dairy Inc., Lafayette, NY.*

Milk produced by high-temperature short-time (HTST) pasteurization is the milk commonly consumed by children in US school lunch programs. Ultra-high temperature direct steam injection (UHT-DSI) without aseptic packaging provides the added benefit of an extended shelf-life, but aseptic (shelf-stable) milk may provide schools systems with a better option. The benefits of making the switch to aseptic milk include shelf stability, non-refrigerated deliveries, and lower storage costs. The objective of this study was to determine child acceptance of unflavored and chocolate 1% milkfat milk processed by 3 different methods: HTST, UHT-DSI non-aseptic, and UHT-DSI aseptic. Unflavored milk was pasteurized at 79°C for 20 s (HTST) or by UHT-DSI at 143°C for 3 s (with or without an aseptic fill). A similar process was used for chocolate milk. Unflavored and chocolate milks processed by the 3 heat treatments were made on 2 different occasions. Milks were evaluated by descriptive analysis (DA) with a trained panel ($n = 7$) to document objective sensory properties. Children who consumed school lunch milk (ages 8 to 12 yr) evaluated the unflavored ($n = 126$) and chocolate ($n = 139$) milks for overall acceptance. Chocolate milks were also evaluated by 2-alternative forced choice (AFC; $n = 30$) before DA and child acceptance testing to confirm equivalent sweetness. Analysis via ANOVA with means separation was applied to evaluate the data. Trained panelists documented sensory differences among the milks processed by different heat treatments ($P < 0.05$). As expected, fewer flavor differences were documented among chocolate milks compared with unflavored milks. Children preferred unflavored HTST milk over UHT-DSI non-aseptic and UHT-DSI aseptic milks ($P < 0.05$). No differences were detected in child overall liking scores between chocolate HTST and UHT-DSI aseptic milks ($P > 0.05$). Chocolate milks processed by UHT-DSI non-aseptic received lower liking scores than HTST chocolate milks ($P < 0.05$). Aseptic milk may be a more viable option than UHT-DSI non-aseptic milk for school meal programs to provide an extended shelf life as well as a product that children may like or accept.

Key Words: children, school milk, aseptic

2080 Drivers of purchase for ready-to-drink coffee latte beverages. R. Adams*, A. R. Best, and M. A. Drake, *North Carolina State University, Raleigh, NC.*

The ready-to-drink (RTD) coffee beverage category is an expanding category with opportunity for both fluid and dried milk ingredients. The objectives of this research were to determine drivers of purchase and important ingredients and label claims for these beverages. An online survey (n = 389) was conducted with RTD coffee beverage consumers. Maximum difference (MXD) scaling and sliding-scale questions as well as Kano analysis and chip allocation exercises were used to assess ingredients and package messaging. Adaptive choice-based conjoint (ACBC) analysis was applied to determine the importance of specific attributes (brand, milk source, flavor, label claim, free-from claim, price) on RTD coffee beverage purchase. Hierarchical Bayes analysis was applied to ACBC and MXD data, followed by agglomerative hierarchical and K-means clustering to segment consumers. Additional survey data were evaluated by univariate and multivariate analyses. Ready-to-drink coffee beverages with milk were preferred by the majority (90%) of consumers. Milk source was the most important attribute, followed by label claim and flavor ($P < 0.05$), and dairy milk was preferred ($P < 0.05$) over a plant-based source. The ideal RTD coffee beverage for all consumers was a national brand in a glass bottle, made with (dairy) milk with “added protein” and organic claims. From the MXD exercise, real ingredients, made with “real milk + cream,” natural, creamy, and high-protein were the most appealing ingredients and label claims ($P < 0.05$). Four unique clusters of consumers were identified. Three of these 4 consumer segments (C2, C3, C4; n = 319) were dairy-focused consumers who desired milk and cream in RTD coffee beverages; dairy-free label claims were negative drivers of purchase for these consumers. Cluster 1 consumers (n = 70) wanted dairy-free and lactose-free label claims and expressed higher concern for climate change and greenhouse gas emissions than other consumers ($P < 0.05$). These results provide a platform for RTD coffee beverage innovation with fluid milk and dairy ingredients.

Key Words: beverage, milk, consumer

2081 Valorizing dairy and poultry byproducts: Bioactive protein hydrolysates with enhanced antimicrobial activity. L. J. Castellanos-Suarez*, O. Campanella, R. Jimenez-Flores, and M. Wick, *The Ohio State University, Columbus, OH*.

Due to their high processing volumes, the dairy and poultry industries produce large quantities of low-value by-products with significant environmental implications, threatening ecosystems. Valorizing these waste streams by recovering crucial nutrients, such as proteins and derived components, offers a sustainable solution to meet the needs of the growing human population. However, the extraction of proteins or protein-derived ingredients, such as peptides, from these byproducts poses challenges, necessitating innovative biotechnological approaches. The objective of this research is to valorize acid whey and mechanically separated turkey (MST) through a combined fermentation. These byproducts, rich in proteins and carbohydrates, offer potential for

sustainable valorization. A series of fermentation treatments were conducted using different combinations of acid whey, MST, molasses, and *Lactobacillus rhamnosus* (OSU-PECh-69). The experimental design was a completely randomized design with 5 treatments and 3 controls. Each treatment was performed in triplicate. Data were analyzed using ANOVA and Tukey’s HSD post-hoc test. Results from fermentation treatments employing OSU-PECh-69 or natural microbiota of these wastes provided insights into hydrolysis yields, produced peptide characteristics, and their molecular weight distribution. Dynamic changes in pH, soluble protein content, and molecular weight over a 10-d fermentation period indicate the transformative nature of the process. Lytic activity against gram-positive bacteria was observed across all treatments in the supernatant and pellet fractions compared with control substrates, whey, or MST. Analysis of antimicrobial activity, using growth inhibition, revealed that the non-neutralized treatments exhibited enhanced antimicrobial activity, with the highest inhibition activity at d 10 with $97.28\% \pm 1.18$ and $99.88\% \pm 1.13$ for gram-negative and gram-positive bacteria, respectively. Results of this study demonstrate the potential of combined fermentation to valorize dairy and poultry byproducts, creating sustainable functional protein hydrolysates for food and feed applications.

Key Words: acid whey, fermentation, antimicrobial activity

2082 Consumer valuation of lactose-reduced ice cream products. C. F. Nicholson¹, G. E. Lewis², E. Krasovskaia³, J. S. Myers^{*1}, and S. A. Rankin¹, ¹*University of Wisconsin–Madison, Madison, WI*, ²*University of Wisconsin–River Falls, River Falls, WI*, ³*Cornell University, Ithaca, NY*.

An estimated 30 to 50 million people in the United States have some degree of lactose intolerance, decreasing their consumption of some dairy foods. Although many lactose-reduced products have been introduced, there is limited information about consumer valuation, which can affect their market success. We implemented an experimental auction to assess consumer valuation for 3 ice cream products (no lactose reduction, lactose-reduced, and lactose-free). The auction comprised 2 rounds of participants (n = 135) bidding on each of the 3 products with an information treatment before the second round describing detailed product characteristics. Random effects censored regression models estimated the influence on bid values of product attributes, additional information, and interactions with the participants’ reported extent of lactose intolerance. Lactose-reduced and lactose-free products increased bid values by \$0.68 and \$1.27 per half-gallon, respectively, for participants reporting any degree of lactose intolerance (Table 1), a 12% to 23% increase over the mean bid value of \$5.46. For participants reporting no lactose intolerance, the effect of products labeled lactose-reduced and lactose-free on bid values was negative (about \$0.60 per half-gallon). The information treatment and its interaction with lactose content had no

Table 1 (Abstr. 2082). Estimated impacts of product and participant characteristics on bid value, US\$ per half-gallon

Variable	Coefficient value	SE	z	$P > z $
Lactose-reduced product	−0.59	0.19	−3.15	0.00
Lactose-free product	−0.61	0.19	−3.24	0.00
Information provided (round)	−0.20	0.14	−1.36	0.17
Lactose-reduced product × information	0.24	0.20	1.19	0.23
Lactose-free product × information	0.28	0.20	1.37	0.17
Lactose-reduced product × any degree of lactose intolerance	0.68	0.20	3.36	0.00
Lactose-reduced product × any degree of lactose intolerance	1.27	0.20	6.25	0.00
Constant	5.46	0.20	27.17	0.00

significant effect at $P < 0.05$. The higher consumer valuation of lactose-reduced and lactose-free ice cream products may indicate opportunities for new product lines by ice cream manufacturers, particularly when complemented with information about changes to make procedures and production costs.

Key Words: consumer valuation, ice cream, lactose

2083 The impact of fat content and water addition on physical and sensory properties of milks. T. Hanks^{*1}, Y. Liu¹, D. M. Barbano², and M. A. Drake¹, ¹*North Carolina State University, Raleigh, NC*, ²*Cornell University, Ithaca, NY*.

Removal of lactose by ultrafiltration plus diafiltration (UF-DF) is an alternative to lactose hydrolysis to make lactose-free milks and milk beverages. In this case, the nonfat, non-protein portion of milk is replaced by DF water at constant protein. Knowledge of the effects of water replacement of the UF permeate portion of milk on sensory and physical properties will assist optimization of sensory properties of milk beverages with lactose removed by diafiltration. The objectives of this study were to determine the impact of water addition on physical and sensory properties of milks at different fat levels. Pasteurized homogenized skim and 12% fat milk were used to standardize milk to 6 fat levels (0%, 2%, 4%, 6%, 8%, and 10% fat). For milk at each fat level, composition, particle size, and fat globule size were measured. Consumer best estimate threshold values (BET) for water addition in each milkfat content were determined at 4°C with and without visual cues using a 7-series 3-alternative forced-choice method ($n = 60$ consumers). For each water addition within a milkfat content, composition, apparent viscosity, color, and freezing point were measured. A trained panel ($n = 7$) evaluated milks with water added at the BET values to characterize the sensory impact of water addition. The experiment was replicated, and ANOVA with mean separation was conducted. Without visual cues, the BET values for water detection in milks decreased when fat content was increased from 0% to 6% (BET values: 11.8% to 5.2%) and increased when fat content was increased from 6% to 10% (BET values: 5.2% to 6.7%; $P < 0.05$). With visual cues, BET values were lower in milk with lower fat content. Milks with water addition generally had lower opacity and viscosity and decreased milkfat and milky flavors, suggesting that visual, aromatic, and texture attributes were all affected, rather than a single modality. These results indicate that removal of lactose by UF-DF will have more influence on sensory properties at lower fat levels, so increasing protein by UF may be important in maintaining the sensory properties and consumer liking of lactose-removed fat-free and low-fat milk beverages.

Key Words: milk, ultrafiltration

2084 Effects of lactose and soluble mineral removal and lactose hydrolysis on heat stability of proteins in ultrafiltered skim milk. D. Hargrove^{*1}, M. A. Drake¹, and D. M. Barbano², ¹*North Carolina State University, Raleigh, NC*, ²*Cornell University, Ithaca, NY*.

Heat-induced protein aggregation and gelation with storage remain challenges for shelf-stable milk beverages. Our objective was to determine the impact of lactose plus soluble mineral concentration and hydrolysis of residual lactose on the heat stability of proteins in skim milk. Pasteurized skim milk (120 kg at 3.5% true protein) was processed by ultrafiltration (UF; 10-kDa cutoff) at 50°C with continuous diafiltration (DF) with deionized water to create a series of skim milks with decreasing lactose and soluble mineral content from 4.77% lactose (skim milk) to 0.03% lactose (final UF retentate). Subsequently, each skim milk in the

series was split into 2 portions, and 1 portion was treated with lactase. Heat stability testing was conducted using an oil bath with a 30-s hold time at 6 temperatures: 135, 140, 145, 150, 155, and 160°C. After heat treatment, the samples were cooled to <4°C, and sedimentable protein, particle size distribution, true protein, nonprotein nitrogen, and pH were determined for heated and unheated skim milks. The experiment was replicated twice. Data were evaluated by ANOVA. As lactose and soluble minerals were removed, both skim milk pH at 20°C and freezing point (FP) increased: pH from 6.67 to 7.34 and FP from -0.511°C to -0.0033°C, respectively ($P < 0.05$). About 93% of the particles in the skim milk were in the size range of casein micelles (0.15 to 0.26 microns). As a result of heating, skim milk pH decreased and protein aggregation increased with increasing temperature and lactose concentration ($P < 0.05$). Protein aggregation and gelation occurred at lower temperatures when lactose and soluble mineral contents were higher. At full lactose and soluble mineral concentration, an increase in protein aggregation and protein sedimentation started at 145°C. Removal of lactose and low molecular-weight soluble solids increased heat stability of skim milk and reduced the browning of milk due to heat exposure. Improved heat stability of milk and milk beverages enables enhanced product consistency and consumer appeal of shelf-stable milk beverages.

Key Words: heat stability, milk protein

2085 Manufacturing mesoscale whey protein particles via a scalable antisolvent precipitation-based method. H. Shi^{*1,2}, O. Velev³, and H. Zheng^{1,2}, ¹*Food Rheology Laboratory, Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, NC*, ²*Southeast Dairy Foods Research Center, North Carolina State University, Raleigh, NC*, ³*Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC*.

The functionality of whey proteins can be broadened and improved by assembling them into mesoscale or microscale particles. This study aimed to manufacture mesoscale whey protein particles (mWPA) using a scalable benchtop method. Aggregation and precipitation of whey proteins occurred within elongated antisolvent streamlines generated by swirl flow in a bulk antisolvent reservoir, controlled by an overhead stirrer. The antisolvent system consisted of 75% ethanol and 25% glycerol. This process resulted in the formation of linear-shaped whey protein assemblies (WPA), which served as precursors to mWPA. A subsequent heat treatment (80°C for 20 min) was applied to strengthen protein-protein interactions and stabilize the WPA structure. Microfluidization (150 MPa for 6 passages) was then performed to reduce the particle size and achieve a more homogeneous particle size distribution in mWPA dispersions. The morphology of mWPA was characterized using transmission electron microscopy (TEM). Particle size and zeta-potential were analyzed using dynamic light scattering and electrophoretic light scattering, respectively. Surface hydrophobicity was evaluated via fluorescent probe labeling, and molecular interactions involved in mWPA were assessed using gel electrophoresis. Additionally, foaming properties, including foam capacity and foam stability at various protein concentrations (0.01%–1%) at pH 6.8 were characterized using a dynamic foam analyzer. The TEM images revealed that mWPA exhibited irregular shapes. At pH 6.8, mWPA had a hydrodynamic diameter of ~197 nm with moderate polydispersity (polydispersity index ~0.26). The zeta-potential of mWPA (-33.27 ± 0.71 mV) was higher than that of untreated WPI (-14.81 ± 0.69 mV; $P < 0.05$), indicating a higher surface charge. Furthermore, mWPA demonstrated higher surface hydrophobicity than untreated WPI at pH 6.8 ($P < 0.05$). Non-reducing and reducing SDS-PAGE analyses confirmed that disulfide bonds were the

primary interactions stabilizing mWPA. Regarding foaming properties, at 0.025% (wt/vol) protein content, the mWPA dispersion exhibited a foaming capacity (foam volume/pumped air volume) at 1.20 ± 0.01 , which was comparable to the untreated WPI dispersion (1.22 ± 0.02). However, after the 900-s aging period, the remaining foam volume ratios were $84.03\% \pm 3.49\%$ and $62.48\% \pm 3.49\%$ for the referred mWPA and WPI systems, respectively. These findings highlight the unique physicochemical properties and superior foam stability of mWPA dispersion compared with untreated WPI, suggesting their potential as functional ingredients for foam-based products at neutral pH.

Key Words: whey protein, whey protein aggregate, antisolvent precipitation, microfluidization, foam capacity, foam stability

2086 Characterization of the plasmin system from skim milk to milk protein concentrate. T. R. Jarrard^{*1,2}, Q. Wu¹, H. Meletharayil³, and H. Zheng^{1,2}, ¹*Food Rheology Laboratory, Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, NC*, ²*Southeast Dairy Foods Research Center, North Carolina State University, Raleigh, NC*, ³*Dairy Management Inc., Rosemont, IL*.

In the manufacturing of milk protein concentrate (MPC), components of the plasmin system such as plasminogen, plasmin, and their activators and inhibitors are concentrated together in the protein fraction. These components may directly or indirectly act against one another in impacting plasmin-driven proteolysis of milk proteins. The effects of processing steps on the activities of plasmin and proteolysis in MPC are not fully understood. In this study, we manufactured MPC85 powder using a pilot-scale membrane ultrafiltration unit and a mini-spray dryer. Protein dispersions with 9% solids were prepared from MPC85 powder and its processing streams (feed milk and liquid retentate). The activities of plasmin in MPC85 and its processing streams were investigated using a fluorescent peptide-coumarin substrate. Additionally, the extent of casein hydrolysis at different aging periods (d 0, 3, 7, and 14 at 37°C) was determined using liquid chromatography. The obtained results indicated that apparent plasmin activities of MPC85 liquid retentate and MPC85 powder were substantially lower than the skim milk feed on a protein basis ($P < 0.05$). However, at the early storage stage (d 3), casein proteolysis in MPC85 powder (~76%) was higher than the feed milk (~67%; $P < 0.05$). After 14 d, casein proteolysis was nearly complete (>90%) for all samples. This initially faster rate of proteolysis in MPC85 powder may be due to the higher substrate (casein) concentration. These data suggest that concentration of milk proteins enhances competitive inhibition in fluorescent plasmin assays, and that the initial rate of casein hydrolysis by plasmin increases with concentration. This knowledge may guide and motivate further research on improving MPC85 quality attributes by reducing its proteolytic potential in diverse food formulas.

Key Words: milk protein concentrate (MPC), plasmin, casein proteolysis

2087 Investigation of mechanical properties of whey protein-stabilized oil-water interfaces during in vitro digestion. Y. Shao^{*} and H. Zheng, *North Carolina State University, Raleigh, NC*.

Bovine whey proteins and their self-assembled aggregates have been studied to stabilize oil-in-water emulsions to deliver micronutrients with poor water solubility. Because gastrointestinal digestion involves intricate reactions at the oil-water (O-W) interface, a thorough understanding of the interfacial behaviors of whey proteins and their assemblies with different structural properties is essential. In this study, we examined

whey protein and 2 distinct types of aggregates derived from it, focusing on their interfacial adsorption dynamics and surface dilatational rheological properties. The 2 whey protein aggregates (WPA-1 and WPA-2) were each manufactured in duplicate by heating a single whey protein isolate (WPI) source at pH 7.0 (9 wt% at 80°C for 24 h) and pH 5.9 (4 wt% at 85°C for 45 min), respectively. Particle size distribution, aggregation percentage, ζ -potential, surface hydrophobicity, protein secondary structure, and colloidal dispersion stability of WPI, WPA-1, and WPA-2 were assessed. The morphologies of WPA-1 and WPA-2 were examined via transmission electron microscopy. Interfacial adsorption dynamics and dilatational rheological properties of each sample were investigated using a Drop Shape Analyzer in which safflower oil and 0.01% wt/vol protein solution were used as the oil and aqueous phases, respectively. The measurements were conducted no less than duplicate. We found that WPA-1 exhibited a smaller hydrodynamic radius (43 ± 3 nm) and a higher percentage of soluble aggregates than WPA-2, which had a radius of 98 ± 2 nm. Both aggregates showed good colloidal stability as well as larger ζ -potential, surface hydrophobicity, and more intermolecular β -sheet than WPI ($P < 0.05$). These differences likely contributed to variations in their interfacial behaviors, influencing the formation and mechanical properties of the protein-stabilized layer at the O-W interface. The WPI had the fastest rate of diffusion-controlled O-W interfacial adsorption (k_{diff}), followed by WPA-1 and WPA-2; however, WPA-2 had a higher rate constant of penetration and unfolding (k_u) than WPI and WPA-1 ($P < 0.05$), indicating that the aggregates undergo more rapid conformational changes upon adsorption, potentially enhancing their interfacial affinity. Regarding the dilatational rheology of protein-coated O-W interfacial layer, WPA-1 tended to exhibit a lower elastic modulus E' than WPI and WPA-2, particularly under small deformations (e.g., 1%–3% area dilatation, $P < 0.05$). Overall, these results suggest that WPA with distinct physicochemical characteristics may result in different O-W interfacial layers with varying rheological behaviors, potentially impacting the emulsion coalescence stability and digestion dynamics. The present work provided fundamental knowledge that serves as a baseline for the next phase of our research, in which we examine how the mechanics of protein-stabilized interfacial films evolve when exposed to simulated digestive fluids. Ultimately, the findings obtained from this study may offer valuable insights into the development of controlled delivery systems.

Key Words: whey protein, oil-water interface, interfacial behavior

2088 The impact of fat and protein contents on bovine milk foaming properties. W. Liang^{*1,2}, H. Meletharayil³, and H. Zheng^{1,2}, ¹*Food Rheology Laboratory, Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, NC*, ²*Southeast Dairy Foods Research Center, Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, NC*, ³*Dairy Management Inc., Chicago, IL*.

Foams originating from whole milk and “half-and-half creamer” are an essential dispersion system being applied in different processed formulas, including coffee and other beverages. However, the effects of milk fat globules (MFGs) on foaming properties are not yet fully understood; this knowledge gap hinders the development of milk foams with superior stability and texture. In the present work, a full factorial design with 3 variables was employed to investigate the impact of composition and temperature on milk foaminess and foam stability. The variables are fat content (0%, 5%, 10%), protein content (3.5%, 5%), and foam holding temperature (25°C, 45°C). The foams were generated using a direct steam injection method. Foamability (V_{10}), defined as the initial foam volume, was ~5.6% higher in the sample with 10% fat and 5% protein,

as compared with the sample with 5% fat and 3.5% protein ($P < 0.05$). Milk foam decay occurred mostly within the first 5–10 min after the foam was made. Foam stability is indicated by the percentage of foam volume reduction in 10 min (V_r). The results showed that, by holding the foam at 45°C, the skim milk foam with 0% fat and 3.5%-protein (V_r : $7.7\% \pm 2.9\%$) was more stable than the high-fat milk foam with 10% fat and 2 levels of protein content (V_r : $12.0\% \pm 1.2\%$ and $12.6\% \pm 3.2\%$; $P < 0.05$). However, this effect was not observed if the foams were held at 25°C. Interestingly, after a 10-min foam holding period, the high-fat milk foams had higher bubble count (BC) and smaller mean bubble radius (R_{average}) compared with skim milk foam ($P < 0.05$). This study showed that enriched fat content (10%) may enhance foamability, and the foam stability is dependent on both fat content and foam holding temperature. The fat-induced foam destabilization may be attributed to air-water interfacial film disruption. The obtained knowledge provides practical guidelines on making milk foams for different coffee and beverage applications.

Key Words: milk foam, milk protein, milk fat

2089 Understanding freezing-induced changes in proteins and fat of Gouda cheese using Fourier-transform infrared and Raman spectroscopy. D. Digvijay^{*1,2}, T. Huppertz^{2,3}, A. L. Kelly², and P. Lamichhane¹, ¹*Food Chemistry and Technology Department, Teagasc Food Research Center, Moorepark, Fermoy, Co. Cork, Ireland,* ²*School of Food and Nutritional Sciences, University College Cork, Cork, Ireland,* ³*FrieslandCampina, Amersfoort, the Netherlands.*

To meet the rising global demand for cheese, especially in milk-deficient countries, and to address the impact of seasonal milk production on cheese quality, frozen storage of cheese has been explored as viable method to stop proteolysis and extend its functional shelf life. However, although freezing and thawing induce structural modifications in proteins and fats at the molecular level, their effects on the texture and functional attributes of cheese are not yet fully understood. Thus, the effects of frozen storage (FrS: -18°C , 3 mo) and rapid freeze-thawing cycles (FTC: 1–5 cycles from 6°C to -18°C to 6°C) on the proteins and lipids in industrial Gouda cheese samples were investigated (in triplicate study) using attenuated total reflectance Fourier-transform infrared (ATR-FTIR) and Raman spectroscopy. Both the freezing treatments resulted in significant ($P < 0.05$) increases in β -sheet and β -turn structures compared with unfrozen control samples, whereas random structures were reduced significantly. However, rapid FTC caused significantly greater increase in β -sheets and decline in random structures than FrS treatment. Raman spectroscopy indicated higher sensitivity for α -helices and β -turns, with notable differences in β -sheets and random coils compared with infrared analysis. Also, repeated (3–5) FTC induced structural stability in the polypeptide backbone, caused fat coalescence, and significantly ($P < 0.05$) increased fluidity in the hydrocarbon chain of lipids. This was supported by an observed increase in the fat pools in FTC (3–5) samples from confocal laser scanning micrographs and a significant ($P < 0.05$) increase in melting and oiling-off properties. Prin-

cipal component analysis of the second derivative of FTIR and Raman spectra indicated differences between FTC and FrS-type treatments. A significant correlation was found between protein conformations and certain textural attributes of cheese, but not with its functional properties. Overall, this study comprehensively highlights the possible role of protein conformations, in addition to factors such as ice and fat crystallization, in the textural changes of freeze-thawed cheeses.

Key Words: cheese freezing, protein secondary structure, texture and functionality

2090 Production of an ingredient rich in MFGM from sweet-cream buttermilk using physico-chemical processes. I. P. Patil^{*} and R. Jimenez-Flores, *The Ohio State University, Columbus, OH.*

The milk fat globule membrane (MFGM) is the membrane enveloping milk fat globules, and is composed of polar lipids such as phospholipids, sphingolipids, glycolipids, cholesterol, and glycoproteins. The MFGM has potential health benefits for gut, brain, mental health, and cognitive functions, among others, across various stages of life. Buttermilk is a by-product of butter production containing water-soluble components from cream and higher-MFGM material than milk and can be used as a valuable source for valorization of MFGM. The objective of this work was to obtain an ingredient from buttermilk rich in MFGM components using physico-chemical processes. Frozen and thawed buttermilk obtained from churning of sweet cream was subjected to change in pH and heat treatment, followed by sedimentation using a benchtop separator. The parameters used for this process were adjusting the pH of buttermilk to pH 5.20, 5.30, 5.40, and 5.60 using lactic acid, then heating to a temperature of 55°C in a water bath, followed by centrifugation. The process was repeated 5 times to obtain the fractions, and statistical analysis was performed using ANOVA at a significance level of 0.05. Sediments obtained from buttermilk processed with pH adjusted to 5.20 yielded the highest quantity of sediment obtained ($6.37\% \pm 2.19\%$) and protein content ($23.59\% \pm 1.08\%$), and the protein and solids contents decreased with increasing pH values. The results from SDS-PAGE showed a higher partition coefficient for precipitation of MFGM proteins in the sediment fractions than that of MFGM proteins in their respective supernatant fractions and buttermilk, when compared at a standardized protein content. At a standardized solids content, phospholipids in the sediments were found to be highest in the sediment from buttermilk with pH adjusted to 5.30 ($0.0972\% \pm 0.0021\%$ phospholipids for sample standardized to 5% solids content), with the concentration of $40.07\% \pm 4.47\%$ more phospholipids compared with the phospholipid content in buttermilk ($0.0675\% \pm 0.0018\%$ phospholipids for sample standardized to 5% solids content). These findings indicate a novel method to obtain ingredients rich in MFGM using physico-chemical treatment of buttermilk, which can be used as functional ingredients for potential health applications.

Key Words: milk fat globule membrane (MFGM), buttermilk, sedimentation

Diversity in Dairy: Posters

2091 Empowering diversity: A panel on women's career development in animal science. H. Hu, M. Farricker*, and T. R. Overton, *Department of Animal Science, Cornell University, Ithaca, NY.*

Women are an integral and growing presence in animal science; yet career paths remain underdefined, hindering leadership and innovation in research and practice. Structural biases and the limited visibility of role models further restrict participation in key sectors. Addressing these issues requires expanding career resources, fostering mentorship, and building inclusive networks. To this end, the Cornell Animal Science Graduate Student Association organized the Women in Animal Science Panel on March 27, 2025, to spotlight women's career development and opportunities in animal science. To shape panelist selection and discussion topics, we surveyed department graduate students (69.2%) and faculty, staff, and postdocs (30.8%) in December 2024. Respondents prioritized research and industry careers (84.6% each), followed by academia (53.8%). Nearly half (46.2%) expressed interest in entrepreneurship and public health, whereas others highlighted farm management, government, consulting, and teaching. Additionally, 76.9% sought insights into career challenges and opportunities. Based

on these results, we selected 6 accomplished scientists with diverse professional backgrounds and lived experiences. Two Cornell faculty participated in person, alongside a government researcher focused on modeling and an industry geneticist in management from other regions. Two additional panelists joined virtually, broadening the discussion to entrepreneurship and non-profit work. This range of geographical and institutional perspectives exposed attendees to varied career pathways and sector-specific challenges, helping them envision their own futures in the field. To ensure an engaging and practical discussion, the panel was moderated by the host of the *Cornell Veterinary Podcast* and incorporated student-submitted questions on career growth and exploration. This initiative not only celebrated the contributions of women in animal science but also actively worked to dismantle barriers to their advancement. By fostering mentorship, representation, and dialog, the panel supported a more inclusive and equitable future for research, education, and industry leadership.

Key Words: women in animal science, diversity and inclusion, career development

Forages and Pastures 1: Posters

2092 The impacts of sward type and inorganic nitrogen fertilization on the rumen metabolome of dairy cows as determined by $^1\text{H-NMR}$. N. J. Suthar^{*1}, E. Wims², R. Pariyani¹, D. Lynch⁴, L. Bateman^{3,4}, A. R. Maguire^{3,4}, J. A. O'Mahony¹, M. Dineen², and T. F. O'Callaghan¹, ¹*School of Food and Nutritional Sciences, University College Cork, Cork, Ireland*, ²*Teagasc, Animal and Grassland Research and Innovation Center, Moorepark, Fermoy, Co. Cork, Ireland*, ³*School of Chemistry, Analytic and Biological Chemistry Research Facility, University College Cork, Cork, Ireland*, ⁴*School of Pharmacy, University College Cork, Cork, Ireland*.

This study examined the effects of pasture management system, specifically sward type and inorganic nitrogen application rate, on the rumen metabolome of spring-calving dairy cows. Twelve rumen-cannulated Holstein Friesian cows were housed indoors and randomly assigned to 1 of 4 dietary treatments, consisting of (1) perennial ryegrass (PRG) receiving 25 kg of inorganic N/ha per cut (PRG-25); (2) PRG receiving 50 kg of inorganic N/ha per cut (PRG-50); (3) PRG-white clover (WC) receiving 0 kg of inorganic N/ha per cut (PRG-WC-0); and (4) PRG-WC receiving 25 kg of inorganic N/ha per cut (PRG-WC-25). Milk and rumen fluid samples were collected, and milk samples were analyzed for composition using ProFoss Milkoscan, whereas rumen fluid metabolome was analyzed using $^1\text{H-NMR}$. Metabolomic profiling revealed that sward type had a greater influence on the rumen metabolome than inorganic nitrogen application rate within treatments. Specifically, cows fed PRG-WC diets exhibited significantly higher concentrations of volatile fatty acids (VFA), including isobutyrate, isovalerate, and valerate, indicating enhanced rumen fermentation. Conversely, cows fed PRG-only diets had increased levels of nucleosides and nucleotides, likely due to controlled microbial growth and increased turnover and nucleic acid release compared with PRG-WC. Other metabolites that were affected by treatment were fumarate and dimethylamine; both were previously demonstrated to have effects on methane emission. Furthermore, Pearson correlation analysis showed that milk fat percentage was correlated with choline ($R = 0.58$), glucose ($R = 0.58$), maltose ($R = 0.54$), glutamate ($R = -0.54$), and nicotinate ($R = -0.55$). Whereas milk yield was correlated with dimethyl sulfone ($R = 0.71$) and choline ($R = -0.63$), milk urea was correlated with VFA, p-cresol ($R = 0.67$), and phenyl acetate ($R = 0.75$). These findings emphasize the strong relationships between diet composition and rumen metabolic processes, highlighting the potential benefits of incorporating white clover into pasture systems to enhance rumen fermentation efficiency.

Key Words: perennial ryegrass, white clover, inorganic nitrogen fertilizer

2093 Evaluation of pasture quality, total mixed ration quality, and voluntary milking system milk yield in A2A2 Jersey herd. T. S. Anthony^{*}, S. M. Brandon, C. Russell, and L. M. Mayo, *North Carolina Agricultural and Technical State University, Greensboro, NC*.

Voluntary milking systems (VMS) continue to gain popularity, allowing farmers to monitor milk production metrics and number of visits sooner on an individual basis. Most VMS systems are enclosed in a barn, and cows do not have access to pasture; however, the combination of 12 h on pasture are limited in research with VMS. The objective of this study was to evaluate the change in pasture quality and total mixed ration (TMR) provided over a growing season in relation to milk yield sampled daily in a VMS. The hypothesis was that milk yield would increase during

times of high-protein pasture and low dry matter content. To test this hypothesis, A2A2 jersey cows ($n = 28$) were fetched to a freestall barn housing the VMS for 12 h each day. Cows were fed ad libitum the same TMR consisting of corn silage (47%), alfalfa hay (4.5%), soybean meal (7.2%), ground corn (8.2%), and a vitamin-mineral premix including Rumensin and Megalac (17.7%) on a dry matter basis. The diet provided 17% crude protein and 0.84 Mcal/kg NEL, including 7 lb as-fed of grain in the VMS. Pastures were a mix of orchardgrass, annual rye, millet, fescue, and small oats. Pastures were measured and sampled before cows entered a new paddock. The TMR was sampled on the same day as pasture sampling. Pasture and TMR samples were submitted for wet chemistry analysis. Statistical analysis included the MIXED and GLIMMIX procedures (SAS Viya, Cary, NC) to evaluate the interactions of pasture height, nutrient content, milk yield, and cow traffic. The MEANS procedure was used to evaluate the changes in pasture height monthly in relation to rainfall. The mean $\pm$ SD pasture height (inches) ranged from 9.3 ± 7.4 in. from June to October, with August being the tallest height consistently (14.6 ± 6.8 in.). The average $\pm$ SD pasture DM was 22.1 ± 6.3 , with the greatest variation being in July (26.4 ± 12.8). The average $\pm$ SD pasture CP was 14.2 ± 2.7 . The milk yield remained constant when averaged by month ($P > 0.05$). In conclusion, despite variations in pasture height, dry matter content, and crude protein levels throughout the growing season, milk yield remained stable.

Key Words: voluntary milking system, milk yield, pasture

2094 The effects of feeding graded levels of hybrid rye silage as a replacement for barley silage on ruminal fermentation characteristics, total-tract nutrient digestibility, and production performance of lactating dairy cows. B. P. Rusawo^{*}, G. B. Penner, and T. Mutsavangwa, *Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada*.

The objective was to evaluate the effects of feeding graded levels of hybrid rye silage as a replacement for barley silage in dairy cow diets on ruminal fermentation characteristics, total-tract nutrient digestibility, and production performance. Twelve lactating Holstein cows (670 ± 68 kg BW; 104 ± 14 DIM) were used in a replicated 4×4 Latin square design with 28-d periods consisting of 18 d for dietary adaptation and 10 d for data and sample collection. Experimental diets were a control diet containing barley silage (46.4% of dietary DM) as the major forage source (CON), and diets containing 15.3% (LOW), 31.1% (MED), and 46.4% (HIGH) hybrid rye silage as a partial or complete replacement for barley silage. Increasing the dietary inclusion of hybrid rye silage linearly decreased DMI as well as the actual and energy-corrected milk yields ($P < 0.01$). Milk fat content was unaffected by diet ($P = 0.87$); however, milk true protein content and milk fat and true protein yields decreased linearly as the dietary level of hybrid rye silage increased ($P < 0.01$). Dietary inclusion of hybrid rye silage linearly increased total-tract starch ($P < 0.01$) and NDF ($P = 0.04$) digestibilities, but DM, CP, and ADF digestibilities were unaffected by diet ($P > 0.05$). Ruminal concentrations of propionate, butyrate, valerate, caproate, and total short-chain fatty acids, and the acetate:propionate ratio, were not affected by diet ($P > 0.05$); however, the ruminal concentration of acetate decreased linearly ($P < 0.01$), whereas those of isovalerate ($P = 0.01$), isobutyrate ($P = 0.02$), and total branched-chain fatty acids ($P = 0.01$) changed in a quadratic manner as the dietary level of hybrid rye silage increased. The ruminal concentration of ammonia-N tended to decrease linearly as the inclusion of hybrid rye silage increased ($P =$

0.07). These results suggest that the partial or complete replacement of barley silage with hybrid rye silage had negative effects on DMI, milk yield, and milk component yields.

Key Words: barley silage, dairy cow, hybrid rye silage

2095 Effect of chopping and plastic film wrapping at baling on the preservation of high-moisture mixed mostly-grass hay. M. G. M. Carvalho^{*1}, R. A. Menezes¹, M. L. Fischer¹, W. Sims¹, M. A. Rahman¹, J. J. Romero², and A. F. Brito¹, ¹University of New Hampshire, Durham, NH, ²University of Maine, Orono, ME.

Dairy farmers may consider the use of high-moisture hay in situations of limited conserved forage availability or inclement weather events. We aimed to investigate the effect of chopping and wrapping on mold and yeast counts, mycotoxin concentrations, and fermentation profile of high-moisture hay (87.2% grass [mostly orchardgrass] and 4% alfalfa) in a completely randomized design experiment with a 2 × 2 factorial arrangement of treatments (n = 6 bales/treatment). Forage was cut at 10 cm above the soil level and left to wilt in the field until harvested as hay at 75% of DM achieved after 76 h of drying. Moisture concentration of hay samples was monitored using portable electric dryers (Koster Moisture Tester). Forage was cut using the round baler knives set with a theoretical chop length of 9 cm to generate the chopped treatment. Knives were removed from the round baler to yield the unchopped treatment before baling. Wrapping of bales was done with 7 layers (25 µm) of plastic film using a round bale wrapper. All bales were stored on wooden pallets in a barn for 75 d before sampling for analysis. Mycotoxins were below detection levels in all hay samples. As expected, total organic acid concentration was below 0.17% (DM basis). The concentration of DM was greater ($P < 0.001$) in unwrapped bales (mean = 83.3%) than wrapped bales (mean = 72.8%). Contrarily, chopping did not affect ($P = 0.72$) the DM concentration of hay. Similarly, treatments did not change ($P \geq 0.38$) the concentrations of CP (mean = 11.1%) and $\text{NH}_3\text{-N}$ (mean = 2% of total N). Mold counts were not affected ($P = 0.98$) by chopping (mean = $5.87 \log_{10}$ cfu/g), but tended to be lower ($P = 0.06$) in unwrapped (mean = $5.48 \log_{10}$ cfu/g) versus wrapped (mean = $6.26 \log_{10}$ cfu/g) hay. In addition, yeast counts were greater ($P < 0.01$) in chopped (mean = $4.89 \log_{10}$ cfu/g) than unchopped (mean = $3.31 \log_{10}$ cfu/g) hay. Yeast counts also were greater ($P < 0.01$) in wrapped (mean = $4.92 \log_{10}$ cfu/g) versus unwrapped (mean = $3.28 \log_{10}$ cfu/g) hay. In brief, no mycotoxins were detected in high-moisture hay despite differences in moisture concentration and mold counts across treatments.

Key Words: forage, hay, preservation

2096 Effects of an ammonium propionate-based preservative on the ruminal in vitro gas production kinetics of high-moisture alfalfa-grass hay round bales. J. Poblete^{*1}, B. Escudero-Alejos¹, M. Chusho-Guevara¹, J. Sandro¹, J. Garzon², T. Schwartz³, T. Ogunjinmi³, E. Neto⁴, E. Rodberg⁴, J. Romero¹, E. Pacanza-Rogers¹, S. Morales¹, and L. Stevens-Rosa¹, ¹Animal and Veterinary Science, University of Maine, Orono, ME, ²University of Maine Cooperative Extension, Orono, ME, ³Chemical and Biomedical Engineering, University of Maine, Orono, ME, ⁴Kemin Industries Inc., Des Moines, IA.

Our objective was to investigate the effects of an ammonium propionate-based commercial preservative on the ruminal in vitro gas production kinetics of alfalfa-grass hay round bales produced at a high moisture concentration ($23.71\% \pm 2.06\%$). The treatments (TRT) were (1) Fresh Cut Plus (FC), a commercial mixture of organic acids (including propionic and acetic acids) buffered with ammonium hydroxide applied

at a 1.2% application rate (fresh basis), and (2) untreated control bales (CON). Twelve large-round bales (244.0 ± 19.7 kg fresh; 1.2×1.0 m; 220 ± 20.8 kg dry matter [DM]/m³) were produced following a randomized complete block design (6 blocks) with areas within the field as the blocking factor. The bales were placed on wooden pallets and stored inside a barn for 60 d. Representative core samples were taken at 0, 5, 15, 30, and 60 d of storage. The ruminal in vitro gas kinetics were recorded for 48 h using the Ankom RF gas production system. Data were analyzed with a 2 TRT × 5 d factorial arrangement with repeated measures using PROC GLIMMIX in SAS v.9.4. Bale initial DM was used as a covariate. Differences were declared significant at $P < 0.05$. In the companion abstract, across all days, FC had (1) lower DM and neutral detergent fiber (NDF) concentration, (2) lower microbial counts (i.e., yeast and molds), and (3) lower internal bale temperature than CON without affecting storage DM losses. At d 15, FC had higher maximum gas production than CON (150.70 vs. 136.85 ± 5.38 mL/g incubated DM), but no differences were observed at the other days ($x = 138.2 \pm 5.38$ mL/g incubated DM). Across all days, no differences between TRT were observed in (1) rate of gas production ($x = 5.20 \pm 0.17\%/h$), (2) lag of gas production ($x = 0.57 \pm 0.15$ h), (3) methane ($x = 12.73 \pm 0.59$ mL/g degraded DM), (4) in vitro true DM digestibility ($x = 68.32 \pm 0.66\%$ DM), (5) in vitro NDF digestibility ($x = 48.47 \pm 1.07\%$ NDF). In conclusion, applying FC increased ruminal gas production at d 15 but did not affect other gas production measures, including methane emissions.

Key Words: methane, digestibility, buffered organic acid

2097 Conventional corn versus short-stature corn: Evaluating the effect of organic acids on fermentation profile and silage stability. H. G. Burchard^{*1}, N. Suresh¹, M. K. Zarrinkar¹, J. Semler¹, T. Fernandes², V. Malacco³, and F. F. Cardoso¹, ¹Department of Animal Sciences, University of Maryland, College Park, MD, ²School of Animal Sciences, Virginia Tech University, Blacksburg, VA, ³Kemin Industries Inc., Des Moines, IA.

Short-stature corn (STC) is a relatively new hybrid with potential as a forage for high-producing dairy cattle. However, further research is needed to validate its effectiveness and forage quality compared with conventional corn (CC). This study aims to evaluate the effects of organic acids on fermentation profile and silage aerobic stability in STC and CC. The treatment groups were as follows: CON (water, no organic acid) and ORG (adding a commercial blend of organic acids; Silage Savor Plus Liquid, Kemin Industries Inc., Des Moines, IA). Experimental silos were used to analyze samples at specific ensiling days (d 7, 15, 30, and 60). Samples were sent to a commercial laboratory (Dairy One, Ithaca, NY). Data were analyzed using the MIXED procedure in SAS. Total acid percentages were greater for CC (8.0%; $P = 0.05$) compared with STC (7.7%), and greater ($P < 0.01$) for ORG (8.4%) compared with CON (7.3%). Lactic acid percentage was greater for CC (5.9%; $P < 0.01$) compared with STC (5.4%) and greater ($P < 0.01$) for CON (5.9%) compared with ORG (5.4%). Acetic acid percentage was greater ($P < 0.01$) for ORG (1.5%) compared with CON (1.3%). Lactic:acetic acid ratio was greater ($P < 0.01$) for CC (4.3%) compared with STC (3.8%) and greater ($P < 0.01$) for CON (4.6%) compared with ORG (3.5%). Ammonia-CPE percentage of CP was greater ($P < 0.01$) for CC (8.5%) compared with STC (7.8%) and greater ($P < 0.01$) for ORG (11.4%) compared with CON (5.0%). Yeast was greater ($P < 0.01$) for STC (2.19×10^3 cfu/g) compared with CC (4.47×10^2 cfu/g) and greater ($P < 0.01$) for CON (2.75×10^4 cfu/g) compared with ORG (3.47×10^1 cfu/g). Aerobic bacteria were higher ($P < 0.01$) for CC (1.41×10^5 cfu/g) compared with STC (9.6×10^4 cfu/g), and there was no difference ($P = 0.56$) between CON (1.1×10^5 cfu/g) and ORG (1.2×10^5 cfu/g).

There was no significant difference in pH levels ($P = 0.09$) between CC (3.85) and STC (3.87), but ORG (3.88) had a higher pH ($P = 0.01$) than CON (3.85). Results indicate organic acids can improve the fermentation profile by increasing total acids for efficient microbial activity and decreasing yeast to aid in silage stability to protect against mold.

Key Words: short stature, fermentation profile, silage stability

2098 The impact of grinding and washing methods and bag preparation on the disappearance of neutral detergent fiber from filter bags used for ruminal degradation. P. Ghimire* and G. Ferreira, *School of Animal Sciences, Virginia Tech, Blacksburg, VA.*

In situ ruminal neutral detergent fiber disappearance (NDFD) is determined by NDF degradation and the loss of particles from bags. This study aimed to evaluate the effect of bag preparation, grinding, and washing methods on the NDFD of forage samples prepared in filter bags for ruminal incubation. Four forages (alfalfa hay, corn silage, grass hay, and triticale silage) were ground using Udy (1 mm; UDY) and Wiley (1 mm; WLY) mills. Ground samples (0.25 g) were placed in filter bags previously rinsed or not in acetone. Duplicate bags for each treatment were prepared and subjected to washing without ruminal incubation. Bags were washed using a laundry machine (MCW, three 3-min cycles), by hand in a bucket (HDW, 1 rinse), or not washed (NOW) before drying at 55°C. Concentrations of NDF in forages and washed residues were determined using α -amylase and sodium sulfite within a chamber (Ankom Fiber Analyzer). The experiment was designed as a randomized complete block design with a $2 \times 2 \times 3$ factorial arrangement of treatments. The model included the fixed effects of grinding, acetone, washing, and their interactions, the random effect of block or forage, and the random residual error. Pairwise least squares means were compared by Tukey's test. Rinsing bags with acetone decreased the NDF loss (7.4% vs. 5.4% NDF loss; $P = 0.02$), and no interactions existed between acetone rinsing and washing methods ($P = 0.82$) or grinding ($P = 0.79$). An interaction existed between washing methods and grinding ($P < 0.01$). Grinding with UDY increased NDF loss relative to WIL under all washing methods ($P < 0.01$), but UDY increased NDF loss to a greater extent with MCW (16.4% NDF loss) than with NOW and HDW (6.9% NDF loss). Grinding samples with WLY resulted in similar NDF losses (2.5%) across all washing methods. No interaction existed between acetone rinse, grinding, and washing methods ($P = 0.52$). In conclusion, grinding, washing methods, and their interactions can account for NDF loss even in samples not incubated in the rumen. This can imply an immediate ruminal degradation of NDF, therefore suggesting the existence of a fraction A in ruminal degradation kinetics.

Key Words: fraction A, lag, kinetics

2099 Impact of grinding and washing methods and bag preparation on in situ ruminal neutral detergent fiber disappearance. P. Ghimire* and G. Ferreira, *School of Animal Sciences, Virginia Tech, Blacksburg, VA.*

In situ ruminal detergent fiber disappearance (NDFD) is determined by NDF digestion and the loss of particles from bags. This study aimed to evaluate the effect of bag preparation, grinding, and washing methods on the in situ ruminal NDFD of forages. Four forages (alfalfa hay, corn silage, grass hay, and triticale silage) were ground using Udy (1 mm; UDY) and Wiley (1 mm; WLY) mills. Ground samples (0.25 g) were placed in filter bags previously rinsed or not in acetone. Duplicate bags were incubated in the rumen of 2 milking dairy cows for 0, 3, 6, 12, and 24 h. After incubation, bags were washed using a laundry machine

(MCW, three 3-min cycles), by hand in a bucket (HDW, one 1-min rinse), or not washed (NOW) before drying at 55°C. Concentrations of NDF in forages and residues were determined using α -amylase and sodium sulfite within a chamber (Ankom Fiber Analyzer). The experiment was designed as a randomized complete block design with a $2 \times 2 \times 3 \times 5$ factorial arrangement of treatments. The model included the fixed effects of grinding, acetone, washing, time, and their interactions, the random effect of block or forage, and the random residual error. Pairwise least squares means were compared by Tukey's test. An interaction existed between acetone and wash ($P < 0.01$). At 0 h, rinsing the bags with acetone increased NDFD with NOW (0.8% vs. 2.3%) and HDW (2.5% vs. 6.4%) but not with MCW (9.7%). At 24 h, rinsing bags with acetone increased NDFD with HDW (8.3% vs. 18.7%) but not with NOW (16.3%) and MCW (29.2%). An interaction existed between grinding and wash ($P < 0.01$). At 0 h, UDY increased NDFD with MCW (3.6% vs. 14.7%) but not with NOW (1.5%) and HDW (4.5%). At 24 h, no differences existed between UDY and WLY for NOW (16.3%), HDW (13.5%), and MCW (24.2%). In conclusion, grinding, washing methods, and rinsing bags with acetone affect NDFD, although acetone rinse does not affect NDFD when using the washing machine. All these artifacts can affect ruminal NDF degradation kinetics, especially at the early stages of incubation. These artifacts could falsely imply an immediate ruminal degradation of NDF or fraction A.

Key Words: fraction A, lag, kinetics

2100 Fungal, nutritional, and mycotoxin composition of alfalfa-grass hay mixtures samples from the Northeast and North Central regions of the United States. B. Escudero Alejos¹, J. B. Poblete¹, M. Chusho Guevara¹, S. L. Annis², G. Pereira³, L. F. Ferraretto⁴, E. C. Diepersloot⁴, J. P. Alvez⁵, Y. Jiang⁴, M. A. Quaassdorff⁶, T. C. Stahl⁷, S. Ishaq¹, H. R. L. Neto⁸, E. Rodberg⁸, and J. J. Romero¹, ¹*School of Food and Agriculture, University of Maine, Orono, ME*, ²*School of Biology and Ecology, University of Maine, Orono, ME*, ³*University of Maine Cooperative Extension, Orono, ME*, ⁴*Department of Animal and Dairy Sciences, University of Wisconsin-Madison, Madison, WI*, ⁵*Center for Sustainable Agriculture, University of Vermont, Burlington, VT*, ⁶*Cornell Cooperative Extension, Batavia, NY*, ⁷*Department of Animal Sciences, School of Environmental and Biological Sciences, Rutgers University, New Brunswick, NJ*, ⁸*Kemin Industries, Des Moines, IA.*

Our objective was to assess the fungal, nutritional, and mycotoxin composition of alfalfa-grass hay mixtures from across the northeast and northcentral regions of the United States. Sixteen hay samples were taken from Wisconsin (8), Vermont (3), Maine (3), New Jersey (1), and New York (1). Samples were collected from distinct hay lots, following National Forage Testing Association protocols. Samples were analyzed with near-infrared spectroscopy for nutritional composition measures and with HPLC-MS/MS for mycotoxin analysis. Fungi were isolated and identified to the genus level by morphological characters. The DM mean was 80.8% (77.0%–85.1%, SD = 3.38), the pH mean was 5.75 (5.12–7.16, SD = 0.509), and the CP mean was 18.1% DM (11.9%–23.4%, SD = 3.03). The mold count mean was 5.62 log cfu/g fresh (4.17–6.72, SD = 0.754), and the yeast count mean was 4.63 log cfu/g fresh (1.5–7.16, SD = 1.374). The mold genera identified were *Phoma* spp. (17 strains), *Aspergillus* spp. (15 strains), *Mucor* spp. (7 strains), *Penicillium* spp. (6 strains), *Cladosporium* spp. (5 strains), *Fusarium* spp. (5 strains), and *Coniothyrium* spp. (1 strain). The most frequently identified genera included *Aspergillus* spp., which was detected in all states except Vermont, and *Phoma* spp., present in samples from all states except New Jersey. Mycotoxin prevalences were as follows: vomitoxin (12.5% of the samples, 0.182–0.221 ppm,

$\bar{x}$ = 0.201, SD = 0.0275), zearalenone (50%, 31.1–414.8 ppb, $\bar{x}$ = 106.7, SD = 126.30), T2 (12.5%, 6.42–51.8 ppb, $\bar{x}$ = 29.1, SD = 32.06), fumonisin B₁ (12.5%, 0.116–0.419 ppm, $\bar{x}$ = 0.294, SD = 0.1582), and fumonisin B₂ (6.3%, 0.112 ppm). In conclusion, *Aspergillus* spp. and *Phoma* spp. were the most frequently identified genera, and zearalenone was the most common mycotoxin. Among the sampled bales, half had DM below USDA-ARS recommendations according to type, size, and weight.

Key Words: hay, mold, mycotoxin

2101 On a molecular basis to reveal interactive association between feed structure and nutrient availability in dairy with cutting-edge synchrotron-radiation molecular microspectroscopy.

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This research program aims to explore how to use cutting-edge synchrotron-radiation molecular microspectroscopic techniques to reveal synergistic impact and interactive association between feed molecular structure and nutrient properties and supply from enzymatic and thermal treated feed tissue and whole grain in dairy cows. The program includes (1) advanced molecular spectroscopic techniques sourced by synchrotron radiation and its application in feeds and nutrition and (2) advanced global-sourced molecular spectroscopy and its application in feeds and nutrition. The emphasis of this study focuses on principle and theory and current applications of cutting-edge molecular microspectroscopy. The global-based feed molecular structure analyses (e.g., DRIFT, ATR-FTIR) are carried out at the Ministry of Agriculture Strategic Research Chair Laboratory at the University of Saskatchewan. The synchrotron-based feed molecular studies in the Ministry of Agriculture Strategic Research Chair (P.Y.) team are carried out at international synchrotron centers, including the Canadian Light Sources (CLS) at the University of Saskatchewan (Canada), the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory in Long Island, New York (US Department of Energy), and Advanced Light Sources (ALS) at Lawrence Berkeley National Laboratory in Berkeley, California (US Department of Energy). The molecular structure spectra statistical analyses, including univariate molecular spectral analysis and multivariate spectral analyses (e.g., PCA, CLS), are carried out using the OMNIC, Unscrambler, and Statistica software. The interactive relationship studies between feed molecular structure and nutrient absorption and delivery are determined using SAS. The program results show a significantly interactive association between feed structure and nutrient availability in animals. The information described in this program gives better insight on use of cutting-edge molecular microspectroscopy to reveal feed internal structure features at cellular and molecular levels that are associated with nutrition utilization in dairy cows. The future research directions are also provided, to advance our current feed molecular structure and nutrition interaction knowledge on both cellular and molecular bases. The results indicate that our animal nutrition model should be developed based on not only feed chemical composition but also on feed inherent structure. In this way, the accuracy of nutrition model could be improved.

Key Words: synchrotron, molecular spectroscopy and applications, nutrition and structure interaction

2102 A comparison of standard and sequential methods of acid detergent fiber (ADF) analysis. G. Ferreira*, *Virginia Tech, Blacksburg, VA*.

Occasionally, the concentration of acid detergent fiber (ADF) in some forages differs minimally from that of neutral detergent fiber (NDF). We evaluated 2 laboratory techniques: a standard ADF method (STD), where forages are directly subjected to ADF analysis, and a sequential ADF method (SEQ), where forages first are subjected to NDF analysis and then to ADF analysis. For this, we used 4 independent samples of alfalfa hay, 4 independent samples of corn silage, and 4 independent samples of triticale silage, analyzing all these samples in 3 independent runs. To determine NDF and ADF concentrations, we used an Ankom200 Fiber Analyzer. For SEQ, we used both α -amylase and sodium sulfite to determine the NDF concentration. After ADF analysis, we determined the concentration of acid detergent lignin (ADL) using an Ankom DaisyII incubator. We performed ANOVA using Proc MIXED of SAS. The model included the fixed effects of the ADF method (STD vs. SEQ), forage group (alfalfa hay vs. corn silage vs. triticale silage) and their interaction, and the random effect of run. The SEQ method of ADF analysis resulted in lower ADF concentrations ($P < 0.01$) than the STD method of analysis (32.0% vs. 27.8%, 19.7% vs. 18.2%, and 41.2% vs. 39.6% for alfalfa hay, corn silage, and triticale silage, respectively). An interaction did not exist between the forage group and the ADF method ($P = 0.40$) for ADF concentration. Similarly, the SEQ method of ADF analysis resulted in lower ADF concentrations ($P < 0.01$) than the STD method of analysis (7.8% vs. 6.5%, 2.1% vs. 0.9%, and 5.8% vs. 3.8% for alfalfa hay, corn silage, and triticale silage, respectively). An interaction did not exist between the forage group and the ADF method ($P = 0.25$) for ADL concentration. A smaller difference between NDF and ADF existed when using the STD method (7.7, 16.4, and 22.2 percentage units for alfalfa hay, corn silage, and triticale silage, respectively) than when using the SEQ method (11.9, 17.6, and 23.9 percentage units for alfalfa hay, corn silage, and triticale silage, respectively). In conclusion, analytical methods can explain the minimal difference between NDF and ADF concentrations of certain forages.

Key Words: acid detergent fiber, alfalfa, sequential

2103 The effect of interseeding kale or sorghum on the yield and forage quality of corn silage in northern New York. A. M. Wilder*, *The William H. Miner Agricultural Research Institute, Chazy, NY*.

Interseeding is being used to control erosion and increase crop species diversity; however, the objective of this study was to evaluate the effects of interseeding on the yield and quality of silage that may contain some of the interseeded crop. Four strips (6 rows wide, 0.76-m row spacing) of corn were interseeded (V4 stage) with forage kale, forage sorghum, or no interseeding treatment in Chazy, New York (silt loam soil). The corn and interseeded crops were allowed to grow together until the corn reached the proper maturity for ensiling. At harvest, each strip was chopped with a multidirectional corn head into a truck. Truck weights were recorded, and 7.6-L bucket minisilos were constructed by filling each bucket to a fixed fill weight. Buckets were ensiled for 90 d before opening and subsampling for quality analysis. Samples were submitted for analysis via both wet chemistry and NIR analyses, and all data were subjected to ANOVA (RCBD, 4 reps) standard least squares model in SASJMP software. No differences were detected in yield, digestion rate (via gas production), fiber, carbohydrates, or mineral content of the silage. The moisture content of the kale treatment silage was 1.5, and 1.3 percentage points lower than the control and sorghum treatments, respectively ($P = 0.02$). Crude protein was 0.45 and 0.41 percentage points lower for the kale and sorghum treatments, respectively ($P = 0.01$). Finally,

crude fat was 0.12 percentage points higher for the sorghum interseeding treatment than the kale interseeding treatment ($P = 0.04$). Because little difference was observed in yield or forage quality, it can be concluded that crops can be interseeded into silage corn without compromising the yield or quality of the resulting silage.

Key Words: interseeding

2104 Evaluation of 18 cover crops for in vitro methane production and digestibility. J. Stypinski^{*1}, D. J. Heuschele², and I. Salfer¹, ¹University of Minnesota, Saint Paul, MN, ²USDA Agricultural Research Service, Saint Paul, MN.

Cover cropping is becoming increasingly popular in dairy production systems, but minimal data exists on rumen fermentation profiles of these crops. Therefore, the objective of this experiment was to evaluate cover crops (CC) for gas production (GP) and composition and dry matter digestibility (DMD). The CC evaluated were barley, buckwheat, camelia, cowpea, crimson clover, golden flax, foxtail millet, kale, yellow mustard, oats, forage pea, pearl millet, daikon radish, Essex dwarf rape, perennial rye, forage sorghum, spring triticale, and spring wheat. Crops were grown in 3 randomized plots per growing season, across 2 growing seasons. The CC were harvested at mid-maturity, dried at 55°C, and ground to 1 mm. One gram of CC was inoculated with 20 mL of rumen fluid and 80 mL of buffer. Rumen fluid was collected from 2 mid-lactation Holstein cows consuming a 50:50 forage:concentrate ration. Bottles were fitted with gas pressure analyzers to record cumulative gas pressure for 48 h. A 10-mL headspace subsample was taken after 48 h for methane concentration (MC) using gas chromatography. Each experimental run consisted of 2 corn silage, 6 randomly selected CC, and blanks. Following 48 h of incubation, fermentation residue was collected, lyophilized, and weighed to calculate DMD. Data were analyzed using a mixed model with fixed effects of crop variety and growing season and random effect of run nested within treatment. Results from a corn silage control were included as a covariate within the model for each parameter to standardize across runs. Total GP (mL) ranged from 82.6 in buckwheat to 139.7 in camelina ($P < 0.01$). Golden flax yielded the lowest MC of 13.2%, whereas fermentation of forage pea and pearl millet yielded the highest MC of 27.5% and 27.2, respectively ($P > 0.01$). Methane production (mL) was lowest for golden flax (11.2%) and greatest for camelina (34.6%; $P > 0.01$), which were primarily driven by MC and total GP, respectively. Yellow mustard yielded the lowest DMD (23.9%) and oats yielded the greatest (61.4%; $P > 0.01$). Cover crops had widely variable GP, MC, and DMD.

Key Words: rumen fermentation, alternative forages, gas production

2105 Extreme mechanical processing of corn and alfalfa silage: Effects on lactation and methane emissions in mid-lactation cows. K. Montes Castillo^{*1}, B. Ollertz-Mertens², K. F. Kalscheur³, E. A. French³, L. F. Ferraretto¹, M. F. Digman⁴, and K. J. Shinnars⁴, ¹Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI, ²Norwegian University of Life Sciences, Ås, Viken, Norway, ³US Dairy Forage Research Center, USDA-ARS, Madison, WI, ⁴Department of Biological Systems Engineering, University of Wisconsin–Madison, Madison, WI.

Techniques that enhance forage fiber utilization are needed to increase the availability of nutrients in dairy cows, resulting in improved lactation performance. Extreme mechanical processing (ExMP) increases the surface area of plant cell walls, potentially improving fiber utilization of forages. We assessed the effect of the ExMP technique on corn and

alfalfa silages, separately and in combination, on lactation performance and methane emissions of dairy cows. Sixty-four mid-lactation multiparous dairy cows (mean $\pm$ SD; 106 ± 29 DIM) were assigned to 1 of 4 diets in a randomized complete block design with a 2-wk covariate period followed by an 8-wk experimental period. Experimental diets were arranged as a 2×2 factorial, evaluating 2 types of corn silage (conventional corn silage and ExMP corn silage) and 2 types of alfalfa silage (conventional alfalfa silage and ExMP alfalfa silage). Forages were processed before ensiling. Methane emissions were measured using GreenFeed units. Data were analyzed using the MIXED procedure of SAS. Dry matter intake (DMI) averaged 29.7 kg/d and was not affected by the type of corn silage nor by the type of alfalfa silage ($P > 0.10$); however, there was a treatment $\times$ week interaction ($P < 0.001$). Milk production averaged 47.9 kg/d and was not affected by treatment; however, there was a treatment $\times$ week interaction ($P = 0.04$). No treatment effect was observed for energy-corrected milk (ECM; 51.6 kg/d); however, there tended to be an interaction of corn silage and alfalfa silage on feed efficiency (ECM/DMI; $P = 0.06$). An interaction of type of silage processed, corn and alfalfa, occurred for fat percentage ($P = 0.03$) and total solids percentage ($P = 0.02$). Milk protein percentage was not affected by treatment ($P > 0.10$). In addition, treatment did not affect milk component yields (kg/d) nor milk urea nitrogen. The use of ExMP forages did not affect BW, BCS, CH₄ production, CH₄ yield, or CH₄ intensity for either silage. These results indicate that ExMP of corn and alfalfa silages did not increase lactation performance or methane emissions of mid-lactation dairy cows.

Key Words: processed forage, lactation performance, methane emission

2106 Effect of 3 maturity stages at harvesting on feed DVE and OEB values in lactating dairy cows from intercropped silages of *Vicia faba* bean and oat plants. C. Nagy, V. Guevara, and P. Yu*, Department of Animal and Poultry Science, College of Agricultural and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada.

The objectives of this study were to determine effect of 3 maturity stages at harvesting on feed DVE and OEB values in lactating dairy cows from intercropped silages made from intercropping whole-plant *Vicia faba* bean cv. CDC Snowbird with whole oat plant cv. CDC Haymaker, evaluated with the Dutch DVE/OEB system. The oat and faba bean plants were intercropped and grown in 3 fields with a seeding ratio of 0.5 bushel of oat grain to 4 bushels of *Vicia faba* bean seeds per acre (or 18 kg of oat seed to 269 kg of faba bean seed per hectare). The intercropped seeding ratio used in this study was chosen based on our preliminary study with different seeding ratios of faba bean seed with oat seeds. The intercropped forages for making silages were harvested at 3 maturity stages: maturity stage 1 was harvested when the oat plants were in the inflorescence stage and the *Vicia faba* bean plants were in the flat pod stage; maturity stage 2 was harvested when the oat plants were in the milk development stage and the faba bean plants were in the milk pod stage; and maturity stage 3 was harvested when the oat plants were at the soft dough stage and the faba bean plants were in the late pod stage. The detailed intercropped silage making conditions, cutting length, particle size, silage pH, VFA profiles, and fresh and dry forage and silage yields have been reported before. The treatment design was a one-way structure. The experimental design was a RCBD with the maturity stages as a fixed effect and fields and fistulated dairy cows for in situ and in vitro studies as random block effects. The results showed that the maturity stage at harvesting affected ($P < 0.05$) total truly digested protein in the small intestine (DVE: ranged from 40.2 to 49.4 g/kg DM) and the degraded protein balance (OEB: ranged from 49.9

to 74.6 g/kg DM), in which all OEB value were positive. These results indicate that there is no shortage of nitrogen in the rumen for microbial protein synthesis from intercropped silages made from 3 maturity stages.

Key Words: intercropping silage, DVE supply, degraded protein balance

2107 Effects of an ammonium propionate-based preservative on the nutrient losses, heating kinetics, and microbial counts of high-moisture alfalfa-grass hay round bales. J. Poblete*¹, B. Escudero-Alejos¹, M. Chusho-Guevara¹, D. Zamudio-Ayala¹, A. P. Jimenez-Lagos¹, M. V. Cardoso¹, A. S. Mindiola¹, J. Sandro¹, S. Annis³, J. Garzon², E. Neto⁴, E. Rodberg⁴, J. J. Romero¹, L. Stevens-Rosa¹, R. Heath¹, ¹Animal and Veterinary Science, University of Maine, Orono, ME, ²University of Maine Cooperative Extension, Orono, ME, ³School of Biology and Ecology, University of Maine, Orono, ME, ⁴Kemin Industries Inc., Des Moines, IA.

Our objective was to investigate the effects of an ammonium propionate-based commercial preservative on alfalfa-grass hay round bales produced at high moisture concentration ($23.71\% \pm 2.06\%$). The treatments (TRT) were (1) Fresh Cut Plus (FC), a commercial mixture of organic acids (including propionic and acetic acids) buffered with ammonium hydroxide applied at a 1.2% application rate (fresh basis), and (2) untreated control bales (CON). Twelve large-round bales (244 kg fresh; 1.2×1.0 m; 220 kg dry matter [DM]/m³) were produced following a randomized complete block design (6 blocks) with areas within the field as the blocking factor. The bales were placed on wooden pallets and stored inside a barn for 60 d. Representative core samples were taken at 0, 5, 15, 30, and 60 d of storage. Data were analyzed as a 2 TRT $\times$ 5 d factorial arrangement with repeated measures using PROC GLIMMIX of SAS v.9.4. Bale initial DM was used as a covariate. Differences were declared significant at $P < 0.05$. After 60-d storage, no differences in storage DM losses were observed between TRT ($x = 2.12\% \pm 1.28\%$). Across all days, FC had (1) a lower DM concentration than CON (78.28% vs. $79.61\% \pm 0.54\%$); (2) lower pH than CON (5.27 vs. 5.80 ± 0.10); (3) a lower neutral detergent fiber concentration than CON (58.66% vs. $60.16\% \pm 0.69\%$ DM basis); and (4) lower mold (5.61 vs. 6.62 ± 0.31 log cfu/g fresh) and yeast counts (3.87 vs. 4.71 ± 0.34 log cfu/g fresh) than CON. During d 5 to d 43 of storage, FC had a lower internal bale temperature than CON (28.06 vs. $40.92 \pm 2.93^\circ\text{C}$). The maximum bale temperature was lower for FC than for CON (32.38 vs. $43.16 \pm 2.33^\circ\text{C}$) during wk 2 to 7 of storage. Similarly, FC had lower heating-degree days ($>30^\circ\text{C}$) than CON during wk 2, 3, 4, and 6 of storage (26.19 vs. $83.80 \pm 17.28^\circ\text{C}$). In conclusion, applying FC minimized the spoilage of alfalfa-grass hay round bales produced at 23.71% moisture by reducing nutrient losses and microbial growth during storage.

Key Words: buffered organic acid, hay spoilage, bale heating

2108 Metabolomic analysis of key off-flavor compounds in a whole-plant corn silage with bitter almond odor. D. Hu, L. S. Zhao, and D. P. Bu*, *Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

Odor is a critical parameter in the sensory evaluation of silage quality. Silage with a bitter almond odor can significantly reduce the feed intake of dairy cows, thereby affecting their production performance and health. This study aimed to analyze the nutritional and fermentation characteristics and key volatile compounds associated with bitter almond odor in whole-plant corn silage. A total of 6 samples were collected, originating from 3 different farms (H, T, and S). From each farm, 3

silage samples with bitter almond odor (K group) and 3 silage samples with normal odor (Z group) were respectively collected and combined to form composite samples. Data for each group and measures were averaged and analyzed using the Mixed procedure of SAS, version 9.4, with $P < 0.05$ considered to indicate statistical significance. The results indicated that there were no significant differences ($P > 0.05$) in the nutritional components and fermentation parameters between the K group and the Z group. Based on electronic nose test, the PCA analysis showed significant differences on sensors W5S and W2W ($P < 0.05$), indicating that specific volatile compounds, such as nitrox and aromatic compounds, as well as sulfur-containing organic compounds, contribute to the unique odor characteristics of silage with bitter almond odor. A comprehensive analysis of the aroma compounds in the K group and Z group was conducted using HS-SPME-GC-MS, identifying a total of 207 volatile compounds. Among these, lipids (73 compounds, 35.27%) and aldehydes (30 compounds, 14.49%) accounted for the majority of the odor components, comprising 49.76% of the total. Differential analysis revealed 38 metabolites that exhibited significant differences. Subsequently, GC-O and odor omission tests are required to reduce the range of key flavor compounds. In summary, understanding the nutrient composition and flavor omics of different odorant silages is expected to establish a scientific foundation for optimizing silage olfactory characteristics and facilitating technological advancements in feed aroma modulation.

Key Words: silage, GC-MS, bitter almond odor

2109 Evaluation of *Megasphaera elsdenii* NCIMB 41125 as an inoculant on the aerobic stability and fermentation characteristics of corn silage and alfalfa haylage. J. Stypinski*¹, S. Traylor², D. J. Heuschele³, and I. Salfer¹, ¹University of Minnesota, Saint Paul, MN, ²Axiota Animal Health, Fort Collins, CO, ³USDA Agricultural Research Service, Saint Paul, MN.

The use of microbial inoculants when ensiling forages is common to reduce the dry matter (DM) losses and increase aerobic stability upon exposure to oxygen. Although previous corn silage (CS) and alfalfa haylage (AH) inoculant work has characterized the effects of lactic acid bacteria, data regarding the effects of CS and AH inoculated with the anaerobic bacterium *Megasphaera elsdenii* are lacking. Therefore, the objective of the current study was to evaluate effects of inoculating CS and AH with a unique strain of *M. elsdenii* on DM loss, aerobic stability, and pH, and organic acid production. Corn silage and AH harvested in central Minnesota in September 2024 and October 2024, respectively. Silages were split into 2 piles each and inoculated with either 500 mL of distilled water (CTRL) or 500 mL of distilled water containing dissolved *M. elsdenii* NCIMB 41125 (Me) at a rate of 6×10^{10} cfu/ton fresh forage. Silage (35% DM) was packed into 12 polyvinyl chloride mini-silos per time treatment and ensiled for either 28 (n = 6/treatment) or 90 d (n = 6/treatment). Initial mass of silos was recorded and compared against mass at time of opening to determine DM losses during ensiling. Upon opening silos, pH and nutrient composition were determined, and aerobic stability was measured by placing silage in a 5-gallon bucket and recording temperature every 10 min for 14 d using wireless temperature monitors (Onset Computer; Bourne, MA). Aerobic stability was determined as time needed increase temperature by 2°C or 3°C . Data were analyzed using a generalized linear model with fixed effects of treatment, ensiling length, and their interaction. Ensiling with Me reduced ($P < 0.01$) DM loss percentage by 60.5% and 59.6% for CS and AH, respectively. Aerobic stability was not affected ($P > 0.40$) by treatment for CS or AH despite Me increasing ($P < 0.01$) pH from

3.82 to 3.88 and from 4.30 to 4.33, respectively. Including Me at time of ensiling would reduce silage waste and feed costs for producers.

Key Words: silage fermentation, forage, mini-silo

2110 Growing degree days as a predictor of pre-harvest quality for Northeastern grass forages. V. L. Blaney¹, M. E. Carabeau¹, E. M. Cabrera¹, E. J. Whittaker^{*1}, M. J. Tetreault¹, and J. A. Winchell², ¹Poulin Grain Inc., Derby, VT, ²Alltech Inc., Lexington, KY.

Measuring the accumulation of growing degree days (GDD) allows farmers to optimize forage harvest timing by predicting crop growth and maturity. The objective of this study was to evaluate the relationship between GDD, harvest timing, and forage quality. A total of 373 grass silage samples were obtained across 29 farms in New England and New York at the time of ensiling, of which 191 were first-cut samples, 113 second-cut samples, and 69 third-cut samples. Data collected included harvest date, field location, and cut intervals. Growing degree days were calculated using the Climate Smart Farming tool with a base temperature of 0°C. For the first cut, accumulation began after March 1; for later cuts, the previous cut date was the starting point. Grass samples were analyzed via near-infrared spectroscopy, and the following parameters were evaluated: crude protein (CP), acid detergent fiber (ADF), organic matter-corrected amylase neutral detergent fiber (aNDFom), NDF digestibility at 12 (NDFD12) and 30 (NDFD30) hours, undegraded NDF at 240 h (uNDFom240), ethanol soluble carbohydrate (ESC), water-soluble carbohydrate (WSC), starch, ether extract (EE), milk per ton, and relative feed quality (RFQ). Statistical analysis was performed using R software (version 4.4.2). Pearson correlation coefficients were calculated to assess the relationships between GDD and forage quality for each parameter, and only significant correlations are reported. For first-cut silages, strong correlations were observed between GDD and ADF ($r = 0.67$), uNDFom240 ($r = 0.81$), CP ($r = -0.67$), NDFD12 ($r = -0.74$), NDFD30 ($r = -0.74$), milk per ton ($r = -0.62$), and RFQ ($r = -0.70$). Moderate correlations were found between GDD and aNDFom ($r = 0.42$) and EE ($r = -0.55$). For second-cut samples, moderate correlations were identified between GDD and ADF ($r = 0.43$), uNDFom240 ($r = 0.48$), and NDFD12 ($r = -0.44$). No significant correlations were found for third-cut samples. In conclusion, GDD accumulation has an influence on forage quality for first-cut grass silages. Collecting agro-

nomics data and earlier third-cut samples may further the use of GDD to optimize forage quality of later cuts.

Key Words: silage harvest, forage quality

2111 Effect of oat variety and oat type (feed type and milling type) on digestive kinetics of crude protein and starch in dairy cows. G. Deng, M. Rodriguez Espinosa, and P. Yu*, *Department of Animal and Poultry Science, College of Agricultural and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada.*

The objectives of this study were to investigate the effects of oat variety and oat type (feed type vs. milling type) on rumen degradation kinetics of crude protein and starch in dairy cows. The oat varieties were provided by the Crop Development Center, including CDC Arborg (milling type), CDC Haymaker (feed and forage type), CDC Nasser (feed type), and Summit (milling type), which were grown and harvested at the university crop research fields for 3 consecutive years. The rumen degradation kinetics of crude protein and starch were determined using in situ technique with fistulated lactating dairy cows. The experiment design was a RCBD in which the data were analyzed using the Mixed model procedure of SAS, with oat varieties as a fixed effect and years and in situ experimental runs as random block effects. Treatment means were compared using Tukey methods. The results showed that the oat variety had significant effect ($P < 0.05$) on rumen degradation rate of protein (K_d , ranged from 2.64%/h in CDC Nasser to 13.33%/h in CDC Haymaker), soluble protein (S, ranged from 10.2% in CDC Arborg to 26.7% in CDC Nasser), and effective degraded crude protein (EDCP, ranged from 64 g/kg DM in CDC Nasser to 102 g/kg DM in CDC Haymaker). For rumen degradation kinetics of starch, the oat variety significantly affected ($P < 0.05$) K_d of starch (ranged from 2.76%/h in CDC Nasser to 15.07%/h in CDC Haymaker), rumen bypass starch (BST, ranged from 121 in CDC Haymaker to 325 g/kg DM in CDC Nasser), and effective degraded starch (EDST, ranged from 151 g/kg DM in CDC Nasser to 312 g/kg DM in CDC Arborg). In conclusion, oat variety significantly affected rumen degradation kinetics of protein and starch in dairy cows.

Key Words: oat variety, feed type and milling type, digestive kinetics, protein and energy

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2112 Proteomic analysis of blood monocytes from dairy cows that were supplemented ex vivo with cannabinoid receptor 2 ligand beta caryophyllene after inflammatory challenge. Y. Butenko¹, B. Shattenstein^{1,2}, P. dos Santos Silva^{1,2}, L. Hubner^{1,2}, and M. Zachut^{*1}, ¹Volcani Institute, Rishon LeZion, Israel, ²Faculty of Agriculture, Hebrew University, Rehovot, Israel.

Activating the cannabinoid receptor 2 (CNR2) in immune cells exerts mostly anti-inflammatory effects. We examined the effects of selective activation of CNR2 by full agonist, β -caryophyllene (BCP), on the proteome of monocytes following an inflammatory challenge ex vivo. Blood monocytes were collected from mid-lactation dairy cows, and were supplemented with BCP at 1 mM or controls. After 24 h, monocytes were challenged with lipopolysaccharide (LPS; 1 μ g/mL), and 6 h later proteins were extracted. We performed proteomic analysis after LPS challenge of protein lysates from monocytes of controls (CTL+LPS) and BCP-supplemented cells (BCP+LPS; $n = 4$ from each treatment) using LC-MS/MS. A total of 3,295 proteins were identified in monocytes; from which 72 proteins were differential by t -test in BCP+LPS vs. CTL+LPS [$P \leq 0.05$ and fold change (FC) ± 1.5], and bioinformatics was performed with Ingenuity pathway analysis software (Qiagen). The protein abundance of prostaglandin-endoperoxide synthase 2 (PTGS2/COX2, $P = 0.009$, FC = 3.0), which is involved in prostanoid biosynthesis, was increased in BCP+LPS vs. CTL+LPS, whereas the abundances of several immune-related proteins were lower; C-type lectin domain family 12 member A (CLEC12A, $P = 0.04$, FC = -1.7), nitrilase family member 2 (NIT2, $P = 0.04$, FC = -4.3), phosphoglycerate mutase 1 (PGAM1, $P = 0.03$, FC = -1.5), as well as interleukin-2 signaling proteins: GRB2 associated binding protein 2 (GAB2, $P = 0.02$, FC = -2.6) and synaptojanin 1 (SYNJ1, $P = 0.02$, FC = -2.4), were lower in BCP+LPS vs. CTL+LPS. Among the top canonical pathways enriched according to the differential proteins were neutrophil degranulation, clathrin-mediated endocytosis signaling, signaling by vascular endothelial growth factor (VEGF), interleukin-2 signaling, and interleukin-3, interleukin-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF) signaling. This is the first proteome data set of monocytes from dairy cows. These results indicate that BCP supplementation ex vivo may affect the response of bovine monocytes to inflammatory stimulus, possibly via CNR2 activation, by modulating the abundances of various prostanoid and immune related proteins.

Key Words: cannabinoid receptor 2, immune, proteome

2113 Endotoxemia induces systemic and adipocyte-specific alterations in oxylipin profiles in dairy cows. M. N. Myers^{*1}, M. Chirivi¹, J. Parales-Girón², J. M. dos Santos Neto², L. C. Worden², A. L. Lock², and G. A. Contreras¹, ¹Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI, ²Department of Animal Science, College of Agriculture and Natural Resources, Michigan State University, East Lansing, MI.

Oxylipins (OXL), bioactive lipid mediators derived from fatty acids, modulate inflammatory responses yet their systemic and tissue-specific dynamics in cows during endotoxemia are unknown. We characterized plasma and adipocyte-specific OXL alterations following LPS exposure in vivo and in vitro. Cows received sterile saline (SAL; $n = 4$) or SAL supplemented with bacterial lipopolysaccharide (LPS, 1 μ g/kg BW; $n = 4$), with plasma collected at baseline, 2 h (+2H), and 12 h (+12H) fol-

lowing infusion. Cultured adipocytes, derived from subcutaneous fat of 4 multiparous cows, were exposed to media or media containing 1 μ g/mL LPS for 3 h. Linear mixed models were constructed for data analysis. Treatment differences reported exceed 20% (all $P < 0.05$). Lipopolysaccharides induced tachycardia, tachypnea, fever, hypersalivation, and reduced feed intake and milk yield. Targeted lipidomics (LC-MS/MS) revealed that LPS increased plasma levels of arachidonic acid-derived, COX-derived TXB₂ and the prostacyclin metabolite 6-keto-PGF1 α , alongside LOX-derived 5-HETE and 15-HETE. Whereas plasma eicosapentaenoic acid-derived, pro-resolving mediators (RvD3, RvD5, PD1) increased at +2H, they transiently declined to basal levels by +12H, suggesting incomplete inflammatory resolution. Lipopolysaccharides upregulated linoleic acid-derived 9- and 13-HODE and CYP-derived 9,10-diHOME at +2H. Lipopolysaccharides increased adipocytes' release of 5-HETE, 6-keto-PGF1 α , 13-HODE, and 19,20-diHDEPA, mirroring systemic changes. Principal component analysis revealed correlations between respiratory rate and plasma RvD5_{n-3,DPA} and 17-HDoHE, and heart rate was positively correlated with 5,6-diHETE and TXB₂. These findings indicate endotoxemia alters systemic and adipocyte OXL profiles, with acute activation of pro-inflammatory pathways and transient pro-resolving responses. Given the potential contribution of adipocytes to systemic OXL release, our results highlight the potential roles of OXL in metabolic and immune regulation during inflammatory challenges. These results provide insight into OXL dynamics during endotoxemia and may serve as biomarkers or targets for the modulation of inflammation in dairy cattle.

Key Words: oxylipin, endotoxemia, inflammation

2114 Use of a direct-fed microbial in early lactation and the effect on innate immune cells following an intramammary LPS challenge. C. Bertens^{*1}, D. Paulus-Compart², C. Stoffel², N. Hogan³, A. Facciolo⁴, and G. B. Penner¹, ¹University of Saskatchewan, Saskatoon, SK, Canada, ²Papillon Agriculture Services, Easton, MD, ³Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada, ⁴Vaccine and Infectious Disease Organization (VIDO), University of Saskatchewan, Saskatoon, SK, Canada.

This study evaluated the effects of feeding a direct-fed microbial (DFM) to cows in early lactation on performance and gene expression of polymorphonuclear (PMN) cells following an intramammary lipopolysaccharide (IMM-LPS) challenge. Multiparous ($n = 28$) and primiparous ($n = 6$) Holstein cows with individual quarter somatic cell count (SCC) $< 250,000$ cells/mL were enrolled at 56 d in milk (DIM). Cows received either 28 g/d of DFM (a blend of probiotics, postbiotics, and exogenous enzymes; DFM) or no DFM (PLB) for 21 d followed by 5 d for DMI and milk measurements. Cows then received either the CON (no challenge) or an IMM-LPS challenge (200 μ g *Escherichia coli* O111:B4 LPS; LPS) into both rear quarters (DFM-LPS, $n = 8$; DFM-CON, $n = 9$; PLB-LPS, $n = 8$; PLB-CON, $n = 9$) on d 27. Blood PMNs were isolated at 0, 24, and 72 h after the IMM-LPS challenge. Data were analyzed using the GLIMMIX procedure of SAS. Data for DMI and milk variables included all 34 cows; whereas PMN gene expression analysis only included cows exposed to IMM-LPS ($n = 16$). Dry matter intake, and milk and milk component yields were not affected by DFM ($P \geq 0.53$), but milk protein concentration tended to be greater for DFM than CON ($P = 0.08$). Feeding DFM reduced PMN abundance in blood by 25% compared with PLB ($P = 0.05$), and IMM-LPS resulted in a 52% and

55% reduction in blood PMN at 24 and 72 h, respectively, relative to 0 h ($P < 0.01$). Direct-fed microbials and LPS interacted to affect NF- κ B expression ($P = 0.04$) such that PLB at 0 h was greater than DFM but not thereafter. Relative gene expression of *SOD2*, *S100A8*, and *S100A9* increased at 24 h compared with 0 h but was not different from 72 h ($P < 0.01$). Expressions of *TLR4*, *IL-1 β* , *CXCR1*, and *STAT3* were down-regulated at 24 h and 72 h compared with 0 h ($P < 0.01$). Expression of *IL-8* was reduced at 24 h relative to 0 h, but not at 72 h relative to 0 or 24 h ($P = 0.02$). There was no effect of LPS on the expression of *IL-10*, *TNF- α* and *TLR2* ($P \geq 0.31$). Overall, the IMM-LPS affected the state of circulating PMN cells; however, other than reduced PMN abundance, the DFM had little effect.

Key Words: blood neutrophil, inflammation, mastitis

2115 Associations of dry-off cow parameters and health and performance during the early lactation period in Holstein dairy cows. A. A. Barragan^{*1}, R. Sorto¹, N. Campos¹, R. Landrum¹, M. Molins², L. Periconi¹, M. Marinelli¹, K. J. Harvatine², T. Ott², and E. Hovingh¹, ¹*Department of Veterinary and Biomedical Sciences, Penn State University, State College, PA*, ²*Department of Animal Sciences, Penn State University, State College, PA*.

The objective of this study was to evaluate associations between cow parameters at dry-off and health, production, and reproductive performance during the early lactation periods in Holstein dairy cows. At dry-off, cows were enrolled ($n = 328$) and several cow parameters were collected (i.e., body condition score, milk yield in the week before dry-off [MKDRY], and lactation). Furthermore, the days from dry-off to the actual calving date (DTC) were calculated after cows calved. Cow lactation data were used to classify cows as primiparous (PRIM) or multiparous (MULT). The body condition score at dry-off was used to group cows in categories (BCSDCT; optimal [OPT] = 2.75–3.5 points; over-conditioned [OVERC] = 3.75–5 points). The MKDRY data were used to group cows in LMKDRY (MKDRY < 32 kg/d) and HMKDRY (MKDRY ≥ 32 kg/d) categories (MKDCT). Health events (≤ 60 DIM), daily milk yield (≤ 112 DIM), and reproductive performance (≤ 120 DIM) data were collected from on-farm computer records. The data were analyzed using the UNIVARIATE, MIXED, GLIMMIX, and LIFETEST procedures of SAS. Overall, multiparous cows had a higher incidence of diseases in the first 60 DIM (MULT = $33.04 \pm 3.35\%$; PRIM = $14.98 \pm 3.57\%$; $P = 0.001$). Also, MULT cows tended to have a higher incidence of mastitis compared with PRIM cows (MULT = $10.39 \pm 2.26\%$; PRIM = $4.22 \pm 2.08\%$; $P = 0.09$). The parameter DTC was associated with clinical metritis and ketosis. Furthermore, the parameters parity, BCSDCT, MKDRY, and DTC, were associated with milk yield in the first 16 weeks after calving. With regards to reproductive performance, only DTC was associated with pregnancy rate at first service and DIM to conception. These results suggest that cow parameters regularly recorded at dry-off could be used to identify groups of cows at risk of developing disease or having poor performance during the early lactation period.

Key Words: dry-off cow parameter, cow health, cow performance

2116 Association of dry-off cow parameters and metabolic status during the dry and transition periods in Holstein dairy cows. A. A. Barragan^{*1}, R. Sorto¹, N. Campos¹, R. Landrum¹, M. Molins², L. Periconi¹, M. Marinelli¹, K. J. Harvatine², T. Ott², and E. Hovingh¹, ¹*Department of Veterinary and Biomedical Sciences, Penn State University, State College, PA*, ²*Department of Animal Sciences, Penn State University, State College, PA*.

This study evaluated the associations between cow parameters at dry-off and metabolic status (i.e., nonesterified fatty acids [NEFA], β -hydroxybutyrate [BHB], and body condition score [BCS]) during the dry and transition periods in Holstein dairy cows. At dry-off, several cow parameters (i.e., BCS, milk yield the week before dry-off [MKDRY], and lactation) were collected from study cows ($n = 155$). The days from dry-off to calving (DTC) were calculated post-calving. Cows were classified by lactation (i.e., primiparous [PRIM]; multiparous [MULT]) and by BCS at dry-off (BCSDCT; optimal [OPT] = 2.75–3.5 points; over-conditioned [OVERC] = 3.75–5 points). Milk samples were collected at dry-off in a randomly selected subgroup of cows ($n = 51$) to measure SCC, and group cows into SCC categories (SCCDCT; LSCC $< 200,000$; [$n = 31$], HSCC $\geq 200,000$; [$n = 20$]). The MKDRY data were used to group cows in LMKDRY (MKDRY < 32 kg/d) and HMKDRY (MKDRY ≥ 32 kg/d) categories (MKDCT). Blood samples were collected daily for the first 5 d and weekly until 21 DIM for assessment of NEFA and BHB. Similarly, BCS was assessed weekly. The data were analyzed using the UNIVARIATE and MIXED procedures of SAS. Parity, BCSDCT, and MKDCT were associated with NEFA and BHB concentration during the first week after dry-off. Interestingly, MULT cows had higher NEFA (MULT = $188.36 \mu\text{mol/L}$, 95% CI: 178.15–199.15; PRIM = $160.62 \mu\text{mol/L}$, 95% CI: 148.01–174.30; $P = 0.002$) and lower BHB (MULT = $562.54 \pm 11.21 \mu\text{mol/L}$; PRIM = $621.92 \pm 17.58 \mu\text{mol/L}$; $P = 0.002$) concentrations compared with PRIM cows. Similarly, OVERC cows had higher NEFA ($P = 0.001$) and had lower BHB ($P = 0.0001$) than OPT cows. On the other hand, HMKDRY cows had higher BHB concentrations ($P = 0.0001$). With regards to NEFA prepartum concentrations, there were associations with SCCDCT ($P = 0.001$), MKDCT ($P = 0.0008$), BCSDCT ($P = 0.09$), and DTC ($P = 0.03$), while only BCSDCT ($P = 0.001$) was associated with postpartum NEFA concentrations. On the other hand, parity ($P = 0.009$), MKDCT ($P = 0.09$), and DTC ($P = 0.01$) were associated with prepartum BHB concentrations, while again, only BCSDCT ($P = 0.02$) was associated with postpartum BHB concentrations. These results suggest that cow parameters that are regularly recorded at dry-off could be used to identify groups of cows that may have altered metabolic status during the dry and transition periods.

Key Words: dry-off cow parameter, metabolic status

2117 Characterizing dairy cow metabolic profile through exhaled breath condensate analysis. M. A. Barrientos-Blanco^{*}, U. Arshad, S. Giannoukos, and M. Niu, *ETH Zurich, Zurich, Zurich, Switzerland*.

The absence of libraries for annotating compounds in the dairy cows' exhalome (breath and ruminal eructation gases) limits metabolic assessment. Secondary electrospray ionization mass spectrometry (SESI-MS) detects numerous exhalome features; however, the mass-to-charge ratio (m/z) output restricts the tentative annotation of compounds. This preliminary study aimed to identify volatile organic compounds (VOC) present in the exhalome of dairy cows. Thirty healthy multiparous lactating Holstein cows (245 ± 51 DIM) were enrolled. Exhaled breath condensate (EBC), representing breath and ruminal eructation VOC, was sampled twice. A head chamber (GreenFeed System; GF) equipped with a gas sampling line was used to collect the exhalome over 12 min/cow. A cold trap system, filled with a mixture of 350 g of dry ice + 200 g of isopropanol, was coupled with the GF sampling line. Samples of EBC were collected when the temperature in the cold trap reached -74°C . The collected samples were: 1) exhalome (Ex) and 2) barn air (environmental background, Bk). Samples from Ex and Bk were

pooled to create one representative sample for each. An untargeted VOC screening was performed using GC-MS. The VOC were identified based on a match factor >70% over the m/z of the referenced feature and ± 15 maximum retention index difference. Features were tentatively annotated using the National Institute of Standards and Technology (NIST) library (NIST17, USA). A metabolite-to-metabolite network interaction analysis was performed using MetaboAnalyst (MA). A total of 507 VOC were identified: 108 unique to Bk, 231 unique to Ex, and 168 present in both Ex and Bk. Only 152 Ex compounds were annotated using the MA algorithm. Isopropyl-alcohol, phenol, and phenylacetaldehyde were found as nodes with the highest degree of interactions. Among relevant metabolites were L-Met and L-Lys linked with isopropyl-alcohol, and oxoglutaric acid and betaine linked to phenylacetaldehyde. Our findings highlight the potential of exhalome analysis as a tool for metabolic assessment and underscore the complexity of compounds identification present in the ruminant exhalome.

Key Words: dairy cow, exhale breath condensate, exhalome

2118 Ceramide synthesis inhibition alters lactation and metabolism in dairy ewes. M. J. Farricker^{*1}, J. L. Judge¹, C. M. Weber², B. C. Tillotson², A. C. W. Lui³, S. Zhang³, M. E. Diaz Rubio⁴, A. N. Davis², and J. W. McFadden¹, ¹*Department of Animal Science, Cornell University, Ithaca, NY*, ²*Biological Sciences Department, State University New York, Cortland, Cortland, NY*, ³*Proteomics and Metabolomics Facility, Cornell University, Ithaca, NY*, ⁴*Rutgers Cancer Institute, New Brunswick, NJ*.

In lactating ruminants, reduced insulin action occurs in muscle to prioritize milk synthesis during a period when ceramide concentrations are elevated. To evaluate ceramide as a potential driver of this response,

lactating ewes were administered myriocin, a serine palmitoyltransferase inhibitor, to inhibit de novo ceramide synthesis (CS) via a continuous intravenous (cIV) infusion. We evaluated 27 formulations via LC-MS/MS to measure myriocin concentrations in different solvents. A stock preparation of 2 mg/mL myriocin in 5% polysorbate (PS)80-saline (MYR) was selected for in vivo testing. Nine East Friesian ewes (1.78 ± 0.83 parity, 81 ± 31.36 d in milk) received one of three 14-d cIV treatments (trt; $n = 3$): 1) CON (5% PS80-saline), 2) LOW (0.015 mg/kg per day MYR), or 3) MOD (0.0375 mg/kg per day MYR). Data were analyzed using a linear mixed model in SAS 9.4. One MOD ewe developed progressive anorexia, with DMI and milk yield (MY) declining over 70% by d 10 (removed from trial). Otherwise, MY and DMI remained stable. The MOD treatment increased milk protein (trt $\times$ d, $P < 0.01$) and lactose yields (trt $\times$ d, $P < 0.01$) as well as ECM (trt $\times$ d, $P < 0.01$). Plasma glucose concentrations increased with time in both MYR groups. LOW induced a sharp nadir in plasma fatty acid concentrations at d 3, while MOD declined gradually, supporting reduced lipolysis from CS inhibition. Liver enzyme elevations (AST, GLDH) in 4 ewes suggest potential PS80-MYR interactions that may have affected metabolism. Plasma ceramides (cer) varied greatly by ewe, especially C22:0-cer (trt, $P < 0.01$). Fold-change (FC) from baseline showed LOW sustained reductions in C22:0-, C24:0-, and total cer (nadir FC: 0.58, 0.63, 0.69 on d 7) with transient reductions in C16:0-, C18:0-, and C20:0-cer. However, we recognize that this may be driven by variation in one ewe on d 0. MOD inconsistently suppressed C16:0- and C24:0-cer on d 7 but remained elevated overall. Unlike cows, LOW-mediated ceramide suppression showed a transient efficiency (MY/DMI) improvement, while MOD remained consistently lower despite elevated ceramide concentrations. Species differences and lactation stage may contribute to these findings, warranting further study.

Key Words: ceramide, myriocin, lactation

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2119 Rumen microbiota for cattle performance and greenhouse gases mitigation across the world. A. Ayemele Gnetegha* and H. Jianlin, *Yazhouwan National Laboratory, Sanya, Hainan, China.*

Studies on rumen microbiota for cattle production and greenhouse gases mitigation are fragmented, and protozoa have been overlooked when evaluating the contribution of microorganisms to cattle sustainability. The present study meta-analyzed the contribution of 3 rumen microbial group (protozoa, bacteria and archaea) to body weight gain, milk yield and to the reduction of methane and ammonia productions. Rumen protozoa, bacteria and archaea were assigned to 80 treatments collected from 4394 papers screened to retain 31 articles, distributed across 15 countries of 4 continents. *Entodinium*, the most predominant rumen protozoa, increased by 24% ($P < 0.05$) at lower (<663 kg) cattle body weight and were maintained ($P \geq 0.05$) at higher body weight (≥ 663 kg). Their abundance increased ($P < 0.05$) by 63% at lower milk yield (<30 kg/d) and decreased ($P < 0.05$) by 36% in higher milk producing cattle (≥ 30 kg/d); this suggests that higher *Entodinium* abundance increased their predation on feed-degrading bacteria, substantially decreasing nutrient bioavailability for higher cattle performance. *Entodinium* abundance decreased by 74% when CH_4 decreased ($P < 0.05$) from 400–650 g/d to 200–400 g/d, associated with high (≥ 100 mg/L) NH_3 production. *Prevotella* was a core bacterial group linked to methane reduction while also playing a key role in nutrient metabolism. Among the archaeal groups, *Methanospaera* and *Euryarchaeota* abundances decreased ($P < 0.05$) with increasing CH_4 content. *Entodinium* is the main rumen protozoa genera that could effectively serve as biomarker for reduction of methane production while increasing cattle body weight and milk yield. This study shed light on the rumen microbial drivers of sustainable and climate-smart cattle production.

Key Words: cattle performance, greenhouse gas, mitigation

2120 More feed efficient cows generate less methane, carbon dioxide, and heat production. S. J. Kendall¹, G. Martinez-Boggio¹, K. M. Kennedy¹, M. J. VandeHaar², F. Peñagaricano¹, K. A. Weigel¹, and H. M. White^{*1}, ¹University of Wisconsin–Madison, Madison, WI, ²Michigan State University, East Lansing, MI.

Animal selection for improved feed efficiency (FE) could improve sustainability through reduced feed cost and potentially by reducing GHG emissions. The objective of this study was to quantify CH_4 , CO_2 , and O_2 emissions and calculate performance metrics based on emissions data in high and low FE cows. Two trials of multiparous mid-lactation Holstein cows ($n = 64/\text{trial}$) were enrolled on an 8-wk study and fed a diet to meet nutrient requirements. Cows were given free access to GreenFeed systems to measure individual cow CH_4 , CO_2 , and O_2 emissions daily. At the end of each study, residual feed intake (RFI) was calculated for each trial individually, then recalculated for the top and bottom 20% RFI cows, with trial included in the model, to identify the most divergent RFI groups ($n = 10$ +RFI [lowFE]; $n = 9$ -RFI [highFE]). Weekly averages of gases were calculated using at minimum 2 records/d, 3 d/wk, and 2 min visit duration. Calculations were conducted as follows: CO_2 fermentation ($\text{CO}_{2\text{Ferm}}$: $\text{averageCH}_4 \times 1.7$), CO_2 respiration ($\text{CO}_{2\text{Resp}}$: $\text{averageCO}_2 - \text{CO}_{2\text{Ferm}}$), respiration quotient (RQ: $\text{averageO}_2/\text{CO}_{2\text{Resp}}$), and heat production {HP: $[16.18 \times \text{averageO}_2 \times (22.4/32.00)] + [5.02 \times \text{averageCO}_2 \times (22.4/44.01)] - [2.17 \times \text{averageCH}_4 \times (22.4/14.03)] - (5.99 \times \text{O}_N)$ }. Final data were analyzed (PROC MIXED, SAS, 9.4) with fixed effect of RFI status and random effect of wk and cow nested within

trial. Data are presented as means [95% CI]; significance is declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. Lower ($P \leq 0.04$) CH_4 (329.82 [266.27, 393.37] vs. 428.85 [364.17, 493.53] g/d) and CO_2 (13,108 [11,987, 14,228] vs. 14,753 [13,606, 15,900] g/d) were emitted from -RFI cows than +RFI cows. Similarly, lower ($P \leq 0.05$) $\text{CO}_{2\text{Ferm}}$ (560.69 [425.65, 668.73] vs. 729.04 [619.08, 839.00] L/d) and $\text{CO}_{2\text{Resp}}$ (12,547 [11,505, 13,588] vs. 14,019 [12,952, 15,086] L/d) were emitted from -RFI cows than +RFI cows. No difference was observed for O_2 or RQ between RFI groups ($P \geq 0.14$). Cows identified as -RFI tended to have lower HP than +RFI cows ($P = 0.10$; 133,473 [120,345, 147,479] vs. 148,200 [136,766, 162,438] kJ/d). These data suggest that more FE cows produce less CH_4 , overall and calculated CO_2 emissions, and HP, improving sustainability.

Key Words: methane, feed efficiency

2121 Development of a facemask system for measuring enteric methane emissions from lactating cows. B. Bae* and C. Lee, *Department of Animal Sciences, The Ohio State University, Wooster, OH.*

We recently developed a facemask system to measure enteric gas emissions in ruminants, which is relatively inexpensive and user-friendly in operation and maintenance. The system measures enteric gas production via spot breath sampling at various time points within a 24-h cycle. The facemask system consisted of a mask, air filter, flowmeter, and air pump, which are connected via tubing (i.d. 3.2 cm). For gas sampling, a Tygon tubing (i.d. 3.2 mm) is connected to the tubing and the other end is connected to a mini air pump. The mini pump continuously subsamples the air flowing from the mask into a sampling bag (3 L). The air flow of the system is about 250 L/min, and the flow of air sampling is about 0.6 L/min. The collected gas sample is analyzed using a photoacoustic gas analyzer for CH_4 and CO_2 concentrations. Daily gas production is calculated by the concentration multiplied by the flow at STP. For validation, a recovery test was conducted 6 times. A standard gas mixture (CH_4 , 60%; CO_2 , 19.5%) was introduced into the mask, and air samples were collected for 2 min. The recovery was 101% (SD, 5.2) for CH_4 and 109% (SD, 1.8) for CO_2 . To determine the optimal time length for breath collection, 4 cows were used, and breath samples were collected from individual cows for 3, 4, or 5 min. The data were analyzed using PROC MIXED of SAS (random, cow; fixed, time). No difference in methane emissions (465 g/d; $P = 0.30$) was found among sampling times, and 3 min was selected. As part of the validation, a diurnal pattern of CH_4 production was evaluated. Eight lactating cows were used, and CH_4 was measured at 16 time points over 6 d (every 1.5 to 2-h sampling in a 24-h cycle). As expected, CH_4 production was 246 ± 25 (SE) g/d before feeding and increased dramatically after feeding (504 ± 17 g/d) and then decreased gradually until the next feeding time (355 ± 37 g/d at 12 h after feeding). In conclusion, the facemask system is a reliable method according to the recovery and pattern of diurnal CH_4 production. Further studies comparing it with other methods (e.g., GreenFeed) are needed to determine its accuracy.

Key Words: enteric fermentation, greenhouse gas, diurnal pattern

2122 Effects of rumen-protected His at different metabolizable protein supplies under adequate Met and Lys on ammonia and greenhouse gas emissions from manure of lactating cows. K. Park^{*1}, J. Kim¹, H. Hu², M. Kambara³, K. Nakagawa³, and C. Lee²,

¹Interdisciplinary Nutrition Program, Department of Animal Sciences, The Ohio State University, Wooster, OH, ²Department of Animal Sciences, The Ohio State University, Wooster, OH, ³Ajinomoto Co. Inc., 1-1 Suzuki-Cho, Kawasaki-Ku, Kawasaki-Shi, Kanagawa, Japan.

This study aimed to investigate the effects of dietary protein level and rumen-protected (RP) His supplementation on CO₂, CH₄, and NH₃ emissions from dairy manure. Forty lactating cows blocked by parity (mean ± SD; 2.43 ± 1.448), DIM (164 ± 26.3), and milk yield (46.6 ± 9.28 kg/d) were randomly allocated to the following diets: diet meeting the requirements of metabolizable protein (MP), Met, and Lys (AMP; 17% CP); diet moderately deficient in MP but adequate in Met and Lys (DMP; 15.5% CP); the DMP diet supplemented with RP-His (DMPH); diet deficient in MP but adequate in Met and Lys (EDMP; 14.6% CP); the EDMP diet supplemented with RP-His (EDMPH). In the last week of the 8-wk experiment, spot feces and urine samples were collected. Individual daily excretion of feces and urine were estimated using indigestible NDF and creatinine concentration, respectively. Manures were reconstituted, and incubated for 12 d using a steady-state air-flux chamber system. Daily emission data were analyzed using PROC MIXED of SAS with fixed effects of diets, day, and their interaction, and a random effect of block. Cumulative gas emissions were analyzed with the model excluding the time effect. Daily feces and urine outputs were not affected by treatments. Daily CH₄ and CO₂ emission rates (mg/cow per day) were not affected by treatments. Cumulative CH₄ emissions (g/cow for 12 d) were not affected by treatments, but RP-His supplementation tended to decrease cumulative CO₂ by 11% ($P = 0.06$; DMPH + EDMPH vs. DMP + EDMP). The daily NH₃ emission rate and cumulative emission decreased linearly ($P = 0.01$) as MP decreased. The daily NH₃ emission rate and cumulative emission were greater ($P < 0.01$) for DMP compared with EDMP or EDMPH. However, those for DMPH were similar to EDMP or EDMPH. In conclusion, lowering the MP supply was effective in decreasing NH₃ emissions from manure. No difference in NH₃ emissions between DMPH and EDMP or EDMPH might reflect improvement in dietary N utilization when additional His was provided with the moderately deficient MP diet.

Key Words: carbon dioxide, methionine, manure incubation

2123 Effects of garlic organosulfides on methane emissions and rumen microbiota in in vitro fermentation. S. Q. Zhang, N. Zheng, J. Q. Wang, and S. G. Zhao*, *Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China.*

Garlic and its extracts, rich in organosulfur compounds, are known to significantly reduce rumen methane emissions. This study explored the relationship between sulfur content and methane reduction by investigating the effects of 3 garlic organosulfides—alliin (C₆H₁₁NO₃S), allicin (C₆H₁₀OS₂), and diallyl trisulfide (DAT; C₆H₁₀S₃)—on in vitro rumen fermentation. The study evaluated greenhouse gas emissions, microbial community composition, feed nutrient degradation rates, methyl-coenzyme M reductase (MCR) activity, and their correlation with sulfur content. Organosulfides were extracted using established methods. Rumen fluid from 3 fistulated Holstein cows (582 ± 24.5 kg) was collected over 5 d. Five batches of anaerobic static cultures (60 mL, 1:2 rumen fluid to buffered inoculum) were prepared, each with 4 systems treated with 2 mM alliin, allicin, DAT, or control. Samples collected at 0, 3, 6, and 12 h were analyzed for gas production (manometer, GC), volatile fatty acids (VFA; GC), ammonia nitrogen (NH₃-N; spectrophotometry), and microbial community (DNA extraction, real-time PCR, 16S rDNA sequencing). At 2 mM, both alliin (86.30% reduction) and DAT (90.08% reduction) significantly reduced methane production ($P < 0.05$), with the extent of reduction positively correlated with sulfur content. Both com-

pounds altered rumen microbiota, reducing fiber-degrading bacteria and fungi ($P < 0.05$). Allicin increased *Ruminococcus albus* and *Prevotella ruminicola* but decreased total fungi and *Fibrobacter succinogenes* ($P < 0.05$). Diallyl trisulfide elevated *R. albus*, *Butyrivibrio fibrisolvens*, *Ruminococcus flavefaciens*, and *P. ruminicola* while reducing total fungi, protozoa, *F. succinogenes*, and *Ruminobacter amylophilus* ($P < 0.05$). These microbial shifts led to decreased fiber degradation, VFA production, and carbon dioxide levels ($P < 0.05$). Additionally, DAT inhibited MCR activity, a key enzyme in methanogenesis ($P < 0.05$). The results demonstrate that garlic organosulfides, particularly those with higher sulfur content, effectively reduce rumen methane production by modulating microbial communities and inhibiting methanogenesis. These findings highlight the potential of garlic-based feed additives to mitigate methane emissions in livestock production, offering a sustainable approach to reducing the environmental impact of agriculture.

Key Words: rumen, methane, garlic organosulfide

2124 Meta-analysis on the effects of grazing management on methane emissions. N. A. Kotey* and R. R. White, *Virginia Tech, Blacksburg, VA.*

Enteric methane (CH₄) produced by ruminants is a leading environmental concern. Given that ruminants are primarily raised on pastures, understanding the effects of grazing management on CH₄ is crucial. However, existing producer-oriented tools for estimating greenhouse gas emissions (GHGe) associated with management practices, such as COMET-Planner, do not provide estimates of CH₄ reductions associated with more intensive grazing systems. Thus, simplified estimates are needed to help producers assess the potential GHGe impacts of their management strategies. This meta-analysis aimed to explore the effects of grazing management on CH₄ from grazing livestock, hypothesizing that more intensive systems, such as rotational grazing, reduce CH₄ emissions compared with continuous grazing. A literature search was conducted between January and July 2024 using Google Scholar. Studies that did not involve experimental research or focused on species other than cattle, sheep, and goats were excluded. This resulted in 46 peer-reviewed experimental studies with a total of 137 observations for the meta-analysis. Methane emissions were recorded in grams per day and grams per unit of dry matter intake, with measurement methods including the sulfur hexafluoride tracer technique, tunnel systems, GreenFeed, and mathematical models. An effect size analysis was performed using linear mixed models in R v 4.4.1 to compare continuous grazing as a control to rotational grazing and management-intensive grazing as treatments. However, only 2 studies with 8 observations met the criteria for the analysis. Univariate and multivariate regressions with CH₄ as the response variable were derived. Performance was evaluated using root mean squared error. Results showed that methane emissions (g/kg DMI) could be 3.6% to 13.6% lower with rotational grazing compared with continuous grazing, depending on species, production stage, forage, and soil type. Although these findings offer valuable insights, the limited number of studies meeting the effect size analysis criteria highlights a need for further research to refine estimates of the effect of grazing management on CH₄ emissions.

Key Words: grazing livestock, methane emission, meta-analysis

2125 Evaluate the precision of enteric methane and carbon dioxide emissions of sniffer method compared to respiration chamber method. N. D. Senevirathne*, D. Alahakoon¹, A. Sweeting², D. Carnovale², and J. W. McFadden¹, ¹Cornell University, Ithaca, NY, ²Agscnt Pty Ltd., Carwoola, NSW, Australia.

Techniques for measuring methane (CH₄) emissions vary in cost and applicability. The Sniffer method provides spot sample emissions for CH₄ and carbon dioxide (CO₂) with high variability compared with respiration chambers (RC). The gold standard for precise CH₄ emission measurements involves housing animals in a RC, which allows for direct measurement of total gas emissions. The use of RC is expensive and labor intensive. Effective strategies to mitigate enteric CH₄ emissions depends on accurate, cost-effective methods to assess emissions from a large number of animals. This study aimed to evaluate the precision and accuracy of a Sniffer method for CH₄, CO₂ and ambient temperature (AT) in comparison to a RC method. Three multiparous lactating Holstein cows (688 ± 28 kg; BW, 29 ± 4 kg milk/d) were assigned in a completely randomized design. Following 3 d acclimation to the facility cows were moved to chambers for 5 d. Cows were offered a TMR (DM basis: 55% corn silage, 12% grass haylage, and 33% concentrate). A Sniffer tool (Agscent [AS]) was placed inside the RC for 5 d to measure CH₄ and CO₂ concentrations, along with AT, enabling simultaneous gas concentration measurements. Data analysis used R packages (stats and lme4) to apply correlation, mean comparison, and regression techniques for CH₄, CO₂, and AT. The mean concentrations of CH₄ and CO₂ were different ($P < 0.01$) between the RC (157 parts per million [ppm], CH₄; 2,445 ppm, CO₂) and AS (161 ppm, CH₄; 2,303 ppm, CO₂). Pearson correlation coefficients for CH₄, CO₂, and AT were 72%, 66%, and 93% ($P < 0.01$), respectively. The mixed-effects linear model, using AS measurements of CH₄, CO₂, and AT, achieved 65% accuracy in predicting CH₄ concentrations compared with the reference conditions in RC. Lin's concordance correlation coefficient showed intermediate agreement for CH₄ and CO₂ indicating 0.72 and 0.63 (95% CI: 0.71, 0.72 [CH₄] and 0.62, 0.63 [CO₂]). Our findings reveal strong correlations between RC and AS when gases are measured simultaneously, highlighting the need for further research to enhance gas measurement prediction models in open barn settings.

Key Words: enteric methane, respiration chamber, sniffer

2126 A meta-analysis of the effects of nitrate supplementation on enteric methane emission, production performance, and methemoglobin in dairy cattle. L. Dicks^{*1}, Y. Roman-Garcia², P. Ingram³, M. Maigaard⁴, S. van Gastelen⁵, and G. F. Schroeder², ¹Cargill Animal Nutrition and Health Innovation Center, Research Centre De Viersprong, Velddriel, the Netherlands, ²Cargill Animal Nutrition and Health Innovation Center, Elk River, MN, ³Cargill Animal Nutrition and Health, Dalton Airfield Estate, Thirsk, North Yorkshire, UK, ⁴Department of Animal and Veterinary Sciences, AU Viborg—Research Centre Foulum, Aarhus, Denmark, ⁵Wageningen University & Research, Wageningen, the Netherlands.

The goal of this meta-analysis was to determine the average and dose dependent responses of nitrate supplementation on methane (CH₄) emission, production performance, and methemoglobin in dairy cows. Eleven publications including 12 studies and 67 treatment means (dose range: 5.3 to 23 g nitrate/kg DM) were used. Mean DMI was 19.5 ± 2.5 kg/d, milk yield (MY) was 27.3 ± 6.7 kg/d, and CH₄ emission was 330 ± 63 g/d (mean ± SD). Data were analyzed using the metafor package in R. Mean difference between control and treatment were estimated using 3 random effects models to account for study variability: 1) a mean model to estimate the overall mean difference across all studies regardless of dose (mean dose: 14.6 g nitrate/kg DM); 2) a model with factor effects of doses centered around the 2 most tested doses: mode dose (MD) of 10.1 ± 1.7 g nitrate/kg DM (n = 21) and higher dose (HD; 20.5 ± 1.8 g nitrate/kg DM, n = 12); and 3) a linear model of the dose response. The overall effect of nitrate on CH₄ was a reduction of 16.3%

($P < 0.01$) compared with control. The second model showed a CH₄ reduction of 15.5% and 17.1% (both, $P < 0.01$) compared with control for the MD and HD, respectively. Although the effect of dose was not significant in the linear model ($P = 0.23$); the model numerically agrees that 10 g nitrate/kg DM decreases CH₄ production by 14.5%. Nitrate reduced DMI by -0.62 ± 0.26 kg/d ($P = 0.02$) in model 1, but model 2 indicated that DMI was reduced only for MD (-0.76 ± 0.33 kg/d, $P = 0.02$). Nitrate did not affect MY or milk fat but reduced protein yield and percent (both, $P < 0.01$) in model 1. In model 2, the MD did not affect MY and milk components, but HD decreased MY, milk protein, and milk fat yield (all, $P < 0.05$). Methemoglobin did not reach levels that pose a risk for animal safety in all studies. In conclusion, at the most common dose of 10 g/kg DM, nitrate reduced CH₄ production by 15.5%. Dose response showed that HD do not significantly increase CH₄ mitigation but negatively affect MY and milk components, compared with MD with no adverse effects on milk production.

Key Words: rumen manipulation, NO₃, methanogenesis

2127 Modernization of dairy farming with zero methane emissions. Nkurikyumukiza^{*}, Icyizere Dairy Farm Center, Kigali, Rwanda.

The project aims at reducing methane gas emitted by livestock especially in dairy farming in Rwanda to sustain smart climate and training Rwandan farmers about adoption of organic farming system to produce quality food approval for human consumption without agrochemical residual in production at farm levels.

Key Words: dairy farming, zero methane, smart climate

2128 Effects of feeding 3-NOP for methane emissions reduction on income over feed costs in the United States. M. R. Pupo^{*}, L. F. Ferraretto, and C. F. Nicholson, University of Wisconsin–Madison, Madison, WI.

Several mitigating strategies to reduce methane emissions have been evaluated, including the inhibitor 3-nitrooxypropanol (3-NOP). Our objectives were to 1) evaluate the effects of feeding 3-NOP in dairy diets on lactation performance and income over feed costs (IOFC) per cow and per farm, and 2) discuss the potential implications for dairy producers. Data from 15 articles met the selection criteria, with a total of 412 lactating cows used. The analysis calculated the mean difference data from the 3-NOP treatment mean minus its respective control treatment mean. The model included 3-NOP supplementation as fixed effect, and study as a random effect. The 3-NOP dose (mg/kg DM) was included as an explanatory variable. Estimate of IOFC (per cow per day) was calculated based on statistically significant ($P \leq 0.05$) changes in DMI, milk yield, and values of milk fat and protein produced less costs for diets feeding 3-NOP. The effects of changes in IOFC were evaluated for herds with different numbers of milking cows. Dairy cows fed 3-NOP had 0.5 kg/d lower DMI and a reduction in milk yield of 0.7 kg/d compared with control cows. Milk fat and protein yields were unaffected for dairy cows supplemented with 3-NOP compared with control. Similarly, the ECM yield and feed efficiency were unaffected for dairy cows supplemented with 3-NOP compared with control. However, a dose of 3-NOP was negatively associated with the 3-NOP effect size on these performance-related parameters. Feeding 3-NOP caused a reduction of 27.9% for CH₄ production at an average 3-NOP dose of 80.3 mg/kg DM. Given changes in DMI, milk yield, milk fat and protein, supplementation of 3-NOP decreased mean IOFC by \$0.35 per cow per day using prices from 2014 to 2023, with a range from -0.21 to -0.42 per cow per day

for a cow producing 39 kg/d. These effects on IOFC suggest that dairy farmers will need to be compensated by participation in carbon credits programs and/or provided other incentives for emissions reduction. Future research is warranted to evaluate long-term effects of feeding 3-NOP on the productivity of dairy cows.

Key Words: 3-nitrooxypropanol, economics, methane

2129 Milk fatty acid profiling for estimating methane emissions in dairy cows: Key predictors and prediction models. E. C. Youngmark*¹ and J. Kraft^{1,2}, ¹*Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT*, ²*Department of Medicine, Division of Endocrinology, Metabolism and Diabetes, University of Vermont, Burlington, VT*.

Milk fatty acid (FA) synthesis and methanogenesis are biochemically linked through rumen fermentation pathways and microbial volatile fatty acid production. Because milk FA composition is known to correlate with methane (CH₄) emissions, FA profiling has been proposed as an indirect method to predict CH₄ emissions from dairy cattle. This study aimed to identify differences in the milk FA profiles of Holstein cows producing either high methane (HM) or low methane (LM) emissions to identify candidate biomarkers for predicting CH₄ output (g/d), CH₄ yield (g/kg dry matter intake), and CH₄ intensity (g/kg energy-corrected milk). Forty-eight cows, fed industry-standard diets based on estimated milk production, were enrolled in a 48-h exploratory trial. Milk samples and CH₄ measurements were collected thrice per day, and intake was recorded daily. The 12 highest and 12 lowest CH₄-emitting cows were identified and sorted into their respective groups. Milk samples were composited by cow and day and analyzed for FA composition via gas-liquid chromatography following lipid extraction and esterification. Methane emission metrics were positively correlated with short- and medium-chain FA, specific polyunsaturated FA, and branched-chain FA, whereas CH₄ emission metrics were negatively correlated with monounsaturated FA, including total 18:1 and 18:1 *cis* isomers. Notably, we observed moderate to strong correlations between several underexplored FA (13:0-*anteiso*; 11-cyclohexyl-11:0; 20:1 *cis*-9; and 20:3 *cis*-5,*cis*-8,*cis*-11) and CH₄ metrics. Regression models integrating milk FA and production parameters effectively predicted CH₄ output ($R^2 = 0.77$ to 0.85), yield ($R^2 = 0.67$ to 0.78), and intensity ($R^2 = 0.77$ to 0.89). These models demonstrated strong alignment between predicted and observed values, underscoring the potential of milk FA profiling as a biomarker-based approach for CH₄ emission prediction. The findings of this study indicate that milk FA may serve as an effective method to detect CH₄ emissions in dairy cattle and highlight the need for further refinement of prediction models.

Key Words: methanogenesis, fatty acid biomarker, GreenFeed

2130 Potential of milk proteomics as a tool for detecting methane emissions in dairy cows. E. C. Youngmark*¹, J. Kraft^{1,2}, and S. L. Greenwood¹, ¹*Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT*, ²*Department of Medicine, Division of Endocrinology, Metabolism and Diabetes, University of Vermont, Burlington, VT*.

Rumen methane (CH₄) production plays a key role in shaping rumen fermentation patterns by promoting fermentation efficiency and influencing microbial activity, nutrient availability, and substrates for milk synthesis. Therefore, changes in rumen methanogenesis could induce shifts in microbial communities, indirectly affecting the milk proteome

by altering the substrates available for protein synthesis. This study investigated differences in the low-abundance milk protein profiles of Holstein dairy cows producing either high methane (HM) or low methane (LM) emissions to identify candidate biomarkers for predicting CH₄ output. Forty-eight cows were enrolled in a 48-h exploratory trial. Milk samples and CH₄ measurements were collected 3 times per day and intake was recorded daily. The highest ($n = 10$) and lowest ($n = 10$) CH₄-emitting cows were identified and grouped accordingly. Milk samples were composited by cow, and the whey fraction was isolated. Isolated proteins were isobarically labeled, fractionated, and analyzed using liquid chromatography-tandem mass spectrometry. Proteomic analysis identified a total of 808 proteins ($n = 20$), with the abundance of 23 proteins affected by emission group. Proteins such as polymeric immunoglobulin receptor (PIgR), peroxisome proliferator-activated receptor δ (PPAR δ), and laminin subunit β -1 (LAMB1) were more abundant in HM cows, while transcobalamin 2 (TC2) was more abundant in LM cows. Principal component analysis revealed distinct clustering of HM and LM cows, driven by proteins correlated with CH₄ emissions. Eighteen proteins showed moderate correlations with CH₄ output, with PIgR exhibiting the strongest positive correlation ($r = 0.65$; $P \leq 0.01$). Proteins, such as PIgR and PPAR δ , LAMB1, and TC2 may reflect microbial activity or volatile fatty acid production in the rumen, underscoring their potential as biomarkers for predicting CH₄ emissions. This study provides new insights into the potential link between CH₄ emissions and milk protein synthesis, highlighting the viability of milk proteomics as an indirect method for estimating CH₄ emissions in dairy cows.

Key Words: low-abundance protein, milk protein biomarker, methanogenesis

2131 Machine learning-based dry matter intake and greenhouse gas emission predictions for lactating Holstein dairy cows. M. Li*, M. Borchers, D. Liang, A. Kulkarni, and F. Di Croce, *Veterinary Medicine Research & Development, Zoetis Inc., Kalamazoo, MI*.

Accurate predictions of dry matter intake (DMI) and greenhouse gas (GHG) emissions in dairy cows is essential for optimizing farm efficiency and sustainability. The objective of this study was to predict DMI (kg/day) and GHG emissions, including methane production (MetP; g/day) and carbon dioxide production (CO₂; g/day), in dairy cows using data from 5 commercial dairy herds. The data set included 82,313 daily measurements from 2,187 Holstein cows collected between June 2022 and February 2024. Data for feed intake and emissions were obtained using SmartFeed and GreenFeed systems (C-Lock Inc.; Rapid City, SD). Daily body weights were recorded using a Walkover Weigh Scale (Data-mars Inc.; Switzerland). Predictions for DMI and GHG were generated using XGBoost, with predictors grouped into 3 tiers based on data availability and ease of collection. Tier 1 (Easy Access) included commonly recorded traits such as week of lactation, lactation number, weather, and feed proximate analysis. Tier 2 (Moderate Access) added milk yield. Tier 3 (Difficult Access) included direct GHG emission measurements, as-fed intake, and body weight. Each higher tier encompassed all lower-tier variables. The data set was divided into 80% training and 20% testing sets randomly by animal, meaning all data from 80% of cows were used for training and all data from the remaining 20% were used for testing. Tier 1, 2, and 3 models achieved strong DMI correlations with measured daily DMI, with coefficients of 0.77, 0.82, and 0.85, respectively. The MetP predictions achieved correlations of 0.50, 0.57, and 0.62 for Tiers 1, 2, and 3, respectively. CO₂ emission predictions yielded correlations of 0.77, 0.78, and 0.82 across the same tiers. These findings demonstrate

the potential of machine learning models to improve DMI and GHG estimation using commercially available farm data.

Key Words: dry matter intake, greenhouse gas emission, dairy cow

2132 Shelf-life stability of bromoform in novel algae-based methane mitigation technologies. D. C. Reyes* and J. W. McFadden, *Cornell University, Ithaca, NY.*

Bromoform supplementation effectively reduces CH₄ emissions in ruminants. However, decreasing retention over time raises concerns about the long-term effectiveness of bromoform-based CH₄ mitigation products. We aimed to investigate the effects of storage temperature and light exposure on bromoform stability in 3 novel formulations: (1) synthetic bromoform (BR-syn), (2) pelleted *Asparagopsis taxiformis* (AT-pellet), and (3) *A. taxiformis* immersed in canola oil (AT-oil) over a 16-wk incubation period. Four storage temperatures (−20, 4, 25, and 37°C) were tested, with fluorescent light exposure assessed at 25 and 37°C. Triplicate samples of each product were stored in clear bags light protected containers. Bromoform content was measured at 7 time points (wk 0, 1, 2, 4, 8, 12, and 16), and iodine content was analyzed at wk 0 and 16. Data were analyzed using one-way ANOVA with Tukey's HSD test. Bromoform retention in BR-syn remained stable across all temperatures (mean = 85.0%) and was unaffected by light exposure ($P > 0.05$). In AT-pellet, retention was highest at −20 and 4°C (92.6%), followed by 25°C (59.5%), with no effect of light exposure ($P > 0.05$). At 37°C, retention significantly declined under light exposure compared with dark conditions (32% vs. 17%; $P < 0.01$). In AT-oil, bromoform retention averaged 92.4% at all temperatures except under light at 37°C, where it decreased to 62.9% ($P < 0.01$). Iodine concentrations remained stable across all treatments after 16 wk, measuring 642, 198, and 35.3 mg/kg for AT-pellet, AT-oil, and BR-syn, respectively. These findings highlight the influence of temperature and light exposure on bromoform stability, particularly in pelleted *A. taxiformis*. The higher stability observed in AT-oil suggests that the oil matrix may protect against oxidation and volatilization, minimizing degradation under high temperatures and light exposure.

Key Words: bromoform, seaweed, stability

2133 Uncertainty of intrinsic-marker-based manure output estimates at farm scale. M. G. Erickson* and E. L. Cortus, *Department of Bioproducts & Biosystems Engineering, University of Minnesota, St. Paul, MN.*

Accurate and precise manure output estimates are critical to modeling environmental impacts from dairy farms and designing manure handling and treatment systems. Through simulations and uncertainty analysis, we examined the potential to use the intrinsic markers acid-insoluble ash (AIA) and undigested neutral detergent fiber (uNDF) to estimate monthly manure output at farm scale. We compiled values reported in the literature to design scenarios representing sand bedding with a uNDF marker (Sand-uNDF) and wood shavings bedding with uNDF and AIA markers (Shavings-uNDF, Shavings-AIA, respectively), with excreta and discharged bedding constituting manure. We assumed daily feed and refusal weights were recorded, bedding usage rates were measured at sampling occasions, and agitated manure was sampled from a transfer pit. Within each scenario, we varied the sampling frequency (weekly, monthly) and sampling and analytical replication (none, triplicate). Then, we propagated input uncertainties to compute the uncertainty of monthly manure output (Δ) at a 95% confidence level relative to the mean. For all scenarios, Δ exceeded $\pm 30\%$ when sampling inputs monthly without

sampling or analytical replication. Weekly sampling without sampling or analytical replication reduced Δ to $\pm 17\%$ to 36% . With weekly sampling of inputs and sampling and analytical triplicates, Δ reached $\pm 10\%$, $\pm 13\%$, and $\pm 34\%$ for Sand-uNDF, Shavings-AIA, and Shavings-uNDF, respectively. In general, uncertainties in feed and manure marker concentrations were the largest contributors to uncertainty in manure output except with Shavings-uNDF, where uncertainty in the mass of bedding also contributed substantially. Considering that few methods currently exist to estimate manure output at farm scale, our results suggest that marker-based estimates may be reasonably accurate and precise with when marker concentrations in bedding are low. Still, more research is needed to examine temporal and sampling variability in outflowing manure in association with management events (e.g., stall cleaning and grooming) and to improve analytical precision for marker concentration.

Key Words: manure, uncertainty, marker

2134 Comparison of in vitro and in vivo methane production from diets varying in starch, unsaturated fat, and fiber. Y. Roman-Garcia*, P. Piantoni, S. El-Haddad, and G. F. Schroeder, *Cargill Animal Nutrition and Health, Innovation Center, Elk River, MN.*

Two experiments were conducted with the objective to compare methane emissions from diets varying in methane production potential between in vivo and in vitro continuous culture (IVCC) systems. In Experiment 1, 12 IVCC fermenters were used in a randomized block design with 2 10-d periods. Fermenters were fed once daily (~80 g). Headspace methane was monitored continuously in all fermenters using a Micro-Oxymax Respirometer (Columbus Instrument Inc., Columbus, OH). In Experiment 2, 10 lactating Holstein cows (35.9 $\pm$ 2.9 kg/d milk yield; 187 $\pm$ 66 DIM; mean $\pm$ SD) were used in a crossover design with 21-d periods. Cows were housed in individual tie-stalls and methane emissions were determined using the GreenFeed system (C-Lock Inc.) at 7 different time points between 0400 and 2000 h over the last 3 d of each period. Experiments were run concurrently and used the same treatment diets: Higher Methane Potential (HMP; 29.2% NDF, 16.0% CP, 25.0% starch, 3.7% fat, and 2.6% unsaturated FA) and Lower Methane Potential (LMP; 27.4% NDF, 15.8% CP, 27.5% starch, 3.8% fat, and 3.0% unsaturated FA). Data were analyzed with the lmer package in R with a model that contained the fixed effect of diet and the random effects of period and experimental unit. For repeated measurements throughout the day, time and its interaction with treatment were also included in the model. In Experiment 1, LMP decreased ($P < 0.01$) methane production by 21% with no time by treatment interaction. In Experiment 2, LMP tended to decrease DMI by 5.2% and energy-corrected milk (ECM) by 4.9% compared with HMT (both $P < 0.10$). The decrease in ECM was through a decrease in the content and yield of de novo and mixed FA and protein in milk (all $P < 0.05$). Compared with HMP, LMP decreased ($P = 0.03$) methane production (g/d) by 8.7%, but did not affect methane yield (13.6 $\pm$ 0.54 g/kg DMI) or intensity (8.49 $\pm$ 0.69 g/kg ECM; both $P > 0.20$). Results confirm that methane production can be reduced by altering the levels of starch, unsaturated fat, and fiber in dairy diets. Furthermore, the degree of reduction was overpredicted (21% vs. 8.7%) by the IVCC compared with in vivo.

Key Words: methane, GreenFeed

2135 Decreasing nitrate doses and their effect on methane production in in vitro continuous culture. S. El Haddad*, Y. Roman-Garcia¹, L. Dicks², E. Margaria¹, L. Wagner¹, and G. Schroeder¹, ¹Cargill Animal Nutrition and Health, Innovation Center, Elk

River, MN, ²Cargill Animal Nutrition and Health, Innovation Center, Veldriel, the Netherlands.

Supplementing nitrate is a well-recognized strategy to reduce enteric methane emissions, with most studies using a standard dose of 1% DM. However, research evaluating responses at lower levels of inclusion is limited. Thus, the objective of this experiment was to evaluate methane production in an in vitro continuous culture (IVCC) system when adding nitrate at lower doses. Twelve IVCC fermenters were used in a randomized block design experiment with two 10-d periods. Treatments were CON (no nitrate), LOW (0.25% DM), MED (0.50% DM), and STD dose (1% DM) of nitrate. Fermenters were fed (~80 g) a grass hay and corn silage-based lactating cow TMR diet once a day (66.3% forage, 40.4% NDF, 13.7% CP). Methane in the headspace was monitored continuously in all fermenters using a Micro-Oxymax Respirometer (Columbus Instrument Inc., Columbus OH). Data were analyzed with the lmer package in R with a model that contained the fixed effects of dose, and the random effects of period and fermenter. For repeated measures time and the interaction with dose were also included. Methane production rate ($\mu\text{mol/h}$) was reduced ($P < 0.01$) by 32% by the LOW treatment, 29% by the MED, and 38% by STD ($\text{SEM} = 27\%$) with no difference among doses. There was a tendency ($P = 0.08$) for an interaction between dose and time which showed the major reduction in methane to be between 2 and 12 h after feeding with no differences at other hours. Total VFA production was not affected by nitrate dose, however adding nitrate increased ($P = 0.05$) acetate proportion and decreased ($P < 0.03$) propionate proportion, which increased ($P < 0.01$) acetate:propionate for all doses compared with CON. No other VFA proportion was affected by treatment. Degradation of NDF numerically increased ($P = 0.19$) when adding nitrate (51% vs. 46%, $\text{SEM} = 4\%$) independently of the dose. Nitrate increased hourly pH at all hours post-feeding compared with CON which could explain the numerical increase in NDF degradation and acetate:propionate. This study validates that nitrate is effective in reducing methane production in vitro and suggests that lower doses of nitrate might be as effective as the current standard dose of 1% DM.

Key Words: methane, nitrate

2136 Organic acid effects on total-mixed ration aerobic stability. L. Garcia^{*1}, E. Rauch¹, V. Malacco², and F. C. Cardoso¹, ¹University of Illinois at Urbana Champaign, Urbana, IL, ²Kemin Industries Inc., Des Moines, IA.

Our objective was to determine whether organic acids applied onto a dry cow TMR with the addition of rye milky silage (55% of total dietary DM) or a fresh cow TMR with the addition of rye boot silage (13.2% of total dietary DM) would improve feed quality by improving aerobic stability measured by monitoring the increase in temperature over time. A total of 12 ($n = 12$) gallon-size buckets were filled with 4 kg of TMR and randomly divided into 4 treatments: dry cow TMR with organic acids (RMUC; Ultra CURB Dry, Kemin Industries Inc., Des Moines, IA; 6 lbs/ton; 46% DM of dietary TMR), dry cow TMR without organic acids (RMWO), fresh cow TMR with organic acids (RBUC; 4 lbs/ton; 42% DM of dietary TMR), and fresh cow TMR without organic acids (RBWO). Recording of TMR temperature began once buckets were filled with their corresponding treatments using data loggers ($n = 2$ per bucket; Thermochron iButtons, iButtonLink, Whitewater, WI) placed in the bottom and top of each bucket. The average temperature for all locations was used. Data loggers were set to 5-min increments and the hourly average for 72 h. In addition, data loggers ($n = 2$) were placed outside of the buckets to register ambient temperature. From Septem-

ber to November, the average ambient temperature was $14.6 \pm 7.9^\circ\text{C}$. Following the 72-h period, data were collected and analyzed using the MIXED procedure in SAS. Least squares means and SE are reported. There was a treatment $\times$ time point interaction ($P \leq 0.05$) in which RMWO had higher temperature (average LSM and SE for these time points [TP]; $33.8 \pm 2.51^\circ\text{C}$) than RMUC ($24.8 \pm 2.51^\circ\text{C}$) starting at TP 23 h up to 37 h and tended ($0.05 < P \leq 0.10$) to be higher from TP 21, 22, and 38 to 48 h. There was no treatment effect ($P > 0.10$) for TMR temperature of a fresh cow diet (RBUC = $20.1 \pm 1.93^\circ\text{C}$ and RBWO = $23.1 \pm 1.93^\circ\text{C}$). In conclusion, the addition of organic acids improved dry cow TMR with rye milky silage by lowering the TMR temperature for a longer period of time, ultimately resulting in a higher quality TMR.

Key Words: rye, temperature, organic acid, total mixed ration

2137 Ensiled fruit waste as sustainable feed for dairy cattle: Effects on productivity and enteric gas emissions. S. Rassler^{*1}, D. Pitta¹, L. Baker¹, Y. Wang^{1,2}, T. Webb¹, J. Bender¹, A. Johnson¹, A. Castenada^{1,3}, K. Narayan¹, A. Post¹, and Z. Dou¹, ¹University of Pennsylvania, Kennett Square, PA, ²China Agricultural University, Beijing, China, ³McGill University, Sainte-Anne-de-Bellevue, QC, Canada.

Culled fruits and vegetables are the largest category of global food waste, contributing significantly to greenhouse gas (GHG) emissions when disposed of via landfills. We propose an innovative approach of ensiling culled fruit as a source of sustainable feed to support livestock production while mitigating climate and resource burdens. The objective of this study was to investigate the effects of replacing 11% and 22% (dry matter basis) of conventional ration ingredients with fruit silage on performance and enteric GHG emissions of dairy cattle. Culled apples were obtained from a local processing center and mixed with grass hay (4:1 as is) to achieve 35% DM, then ensiled in individually wrapped bales. Twenty-four lactating Holstein cows blocked by parity, milk production, and days in milk were randomly assigned to 3 groups: control (0% fruit silage), T11 (11% fruit silage inclusion), and T22 (22% fruit silage inclusion). A 21-d adaptation period was followed by a 21-d experimental period, during which individual dry matter intakes (DMI) using Calan tubs (American Calan Inc., Northwood, NH), milk yield and components (Afimilk, Kibbutz Afikim, Israel) and enteric gaseous composition using GreenFeed (C-Lock Inc., Rapid City, SD) were measured. Results showed no significant differences between treatments for DMI (kg DM/d), milk yield (kg/d), fat (%), protein (%), or enteric methane emissions (g/d). However, feed efficiency (milk yield/DMI) differed by treatment, week, and their interaction ($P < 0.01$). Interestingly, there was more enteric H_2 (g/d) and higher ($P < 0.01$) total volatile fatty acid concentrations for T22 compared with control. The absolute concentration (mM) of acetate was lower, and butyrate was higher for T22 compared with control, whereas no differences were observed for T11 versus T22. In conclusion, coensiling culled fruit waste with grass hay is a viable option for feed preservation and landfill diversion. The ensiled product may be incorporated into lactating cow diets up to 22% without disrupting performance or GHG emissions, thereby positively enhancing circular and sustainable dairy production.

Key Words: fruit waste, ensiling, methane

2138 Exploring color spaces to improve the use of image analysis for assessing total mixed ration uniformity. I. G. M. A. Santos^{*1}, A. R. Reibman², and J. P. Boerman¹, ¹Department of Animal Sciences, Purdue University, West Lafayette, IN, ²School of Electrical and Computer Engineering, Purdue University, West Lafayette, IN.

The use of image analysis has emerged as a powerful tool in precision livestock farming; however, challenges such as the presence of shadows and inconsistent lighting conditions need to be overcome to optimize the use of image analysis. The objective of this study was to explore the use of different color spaces to minimize the effects of shadow on image analysis as a tool to assess total mixed ration (TMR) uniformity. Initially images were assessed using the red, green, blue (RGB) color space; however, RGB is highly sensitive to variations in lighting and shadows, leading to high variability in uniformity measurements. To explore other alternatives, we analyzed the HSV color space, which separates image into 3 distinct components: Hue (H), representing the dominant color type; Saturation (S), which measures color intensity; and Value (V), which indicates brightness. This separation makes it possible to assess their robustness against lighting variations. Using recorded videos of the TMR mixing process, the initial and final frames of 9 batches across 3 d were analyzed. In each frame, 100 regions of interest (ROI) were selected, and pairwise comparisons were conducted using the Earth Mover's Distance (EMD) to quantify uniformity. The diets used were considered uniform based on other measurements of uniformity. Normalization was performed using the mean EMD value of all initial frames within each color space to allow cross-space comparisons, using *t*-tests compared with the RGB results. Results showed that the H channel significantly reduced variation compared with RGB ($P = 0.005$, mean difference = -0.189), with lower EMD values, indicating that H better captured uniformity with reduced sensitivity to lighting variations. The S channel exhibited significantly higher EMD values than RGB ($P = 0.001$, mean difference = 0.135), suggesting that it amplified differences rather than minimizing them. The V channel did not significantly differ from RGB ($P = 0.37$, mean difference = -0.003), indicating that brightness did not influence uniformity measurement in a meaningful way. Overall, the H channel performed the best in minimizing lighting effects, indicating that it may be an effective color space to use to minimize the effect of shadow when evaluating video or images.

Key Words: color channel, total mixed ration, shadow

2139 Variability of water intake in dairy cows for the assessment of water efficiency. A. Costa^{*1}, M. Lamanna¹, D. Cavallini¹, M. Bovo², R. Colleluori¹, and G. Visentin¹, ¹Department of Veterinary Medical Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy, ²Department of Agricultural and Food Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy.

Feed efficiency and precision feeding strategies are essential to reduce the environmental impact of dairy and beef sector. Water is a precious resource, and because cattle are far from being considered as a water-neutral livestock, identification of superior animals is advisable. Water efficiency can be described by the amount of water ingested per kg of milk or meat produced. In this study, we investigated phenotypic effects affecting daily water intake (WI) and water efficiency index (EFF₁) calculated as ratio of WI to milk yield (MY). Similarly, the EFF₂ was calculated by using WI predicted by reticular boli manufactured by smaXtec animal care GmbH. Twenty-four Holstein cows with bolus inserted, 7 primiparous and 17 multiparous, monitored for 28 d (Jan.–May 2024) at the University of Bologna experimental farm. Every day individual WI at each bout and MY at each milking were recorded through in-house developed drinkers. To study the phenotypic variability of the traits we used a linear mixed model where cow was the random effect and parity (1, ≥2), lactation stage (≤80, 81–100, ≥101 d in milk) and their interaction were fixed. Measured WI and predicted WI averaged 183.0 and 176.3 L/d. Primiparous drank less than multiparous (155.4 vs. 194.2 L/d; $P < 0.001$) and WI increased with days in milk (166.0,

175.8, and 182.5 L/d; $P < 0.001$). As regards EFF₁, primiparous were the most efficient (3.4 vs. 3.8 L/kg milk; $P < 0.001$) as well as cows in ≤80 d in milk (3.2 L/kg milk; $P < 0.001$). None of the fixed effects were significant for EFF₂, although trends for parity and lactation stage were numerically similar to EFF₁. The Pearson correlation between EFF₁ and EFF₂ was 0.49 ($P < 0.001$) and that between measured and predicted WI was 0.24 ($P < 0.001$). MY was significantly correlated with both EFF₁ (-0.44) and EFF₂ (-0.46). Results indicate that WI is variable and, at the same time, suggest that there is potential to select water-efficient cows without compromising MY. Further research may explore genetic aspects and inclusion of WI in the definition of feed efficiency. Finally, sensors for large-scale accurate phenotyping of WI are also needed.

Key Words: water use, precision farming, natural resources depletion

2140 Association between estimated dry matter intake dynamics and time to conception of Holstein cows. R. Champion^{*}, J. Vargas, P. Pinedo, and D. Manriquez, Colorado State University, Fort Collins, CO.

Reproductive performance in lactating dairy cows is closely linked to their energy balance. This study aimed to estimate individual DMI (eDMI) using daily body condition score (BCS) and milk yield data as proxies for energy input and output, respectively, and to assess the association between eDMI and time to conception. This retrospective observational study used data from 12,048 lactations from 7,260 Holstein cows housed in a commercial dairy farm in Colorado, calving between April 2019 and January 2022. The BCS (1–5 points, 0.1 increments) data were generated daily by an automated BCS camera system (DeLaval, Sweden) located at the exit of the milking parlor. Milk yield (lb/d per cow) data were collected from the farm milking machine software (Delpro, DeLaval). Equations from the NRC and NASEM were used to calculate individual daily eDMI using BCS, milk FCM, reference body weight, requirements for maintenance, and lactation information. Monthly averages of eDMI, BCS, and milk yield values were obtained for each study cow. Reproductive information was retrieved from the farm management records software (DairyComp, VAS), and conception was categorized as early conception (EC, ≤150 DIM), late conception (LC, >150 DIM), and no conception (NC). Mixed models were used to assess the relationship between eDMI and the conception category. Model fit statistics were considered to evaluate the performance of the eDMI equations. A total of 8,101 cows were classified as EC, 1,994 as LC, and 1,953 as NC. The NC cows had greater eDMI for lactation mo 1, 2, and 3 (8.35 ± 0.03 ; 11.20 ± 0.03 ; 11.97484 ± 0.18 kg/d) compared with EC (8.12 ± 0.01 ; 10.93158 ± 0.01 ; 11.757114 ± 0.01 kg/d) and had greater eDMI for mo 1 and 2 compared with LC (8.17 ± 0.27 ; 11.11 ± 0.27 ; 11.93 ± 0.27 kg/d). At mo 4 LC (12.29 ± 0.27 kg/d) had greater eDMI compared with EC (12.11 ± 0.14 kg/d) and NC (12.11 ± 0.41 kg/d) and tended to remain greater until mo 10 of lactation. This approach allows for the characterization of individual and group DMI using information available from precision technologies and milk equipment and might be used to establish monthly eDMI associated with specific reproductive outcomes.

Key Words: automated body condition, intake, reproductive performance

2141 A new approach to data mining electronic feeder data for individual cattle monitoring. A. S. C. Maia^{*1,3}, J. B. Hall², and I. M. A. Teixeira³, ¹Sao Paulo State University, Jaboticabal, SP, Brazil, ²University of Idaho, Carmen, ID, ³University of Idaho, Twin Falls, ID.

Precision livestock farming (PLF) has introduced a variety of commercial solutions to dairy cow management, including electronic feeders (EF) integrated with radio frequency identification (RFID) to monitor daily individual feed intake in real-time. However, data from such systems can be biased due to issues such as when an animal leaves the automated feed bunk, but the RFID tag continues to register the animal's presence, possibly due to a malfunctioning RFID reader. This results in the need for data cleaning to eliminate inaccurate records. This study aimed to propose a novel algorithmic approach for mining electronic feed intake records to improve the analysis of feeding behavior. The data analyzed were collected from 2,684 cattle over 20 experimental trials (days on feed = ± 70 d) conducted at the Nancy M. Cummings Research Extension & Education Center (University of Idaho, Carmen, Idaho, USA) between March 2013 and April 2024. Each study used 4 pens equipped with 6 electronic feeders (GrowSafe), and each feeding event was recorded by RFID, capturing the animal's presence at the feed bunk, date, time, and amount of feed consumed. The data mining approach developed in this study involved 5 key steps: (1) identifying meal events and their durations, (2) detecting inconsistencies, errors, and outliers, (3) addressing these inconsistencies using machine learning models, (4) identifying deviations from feed intake trends in relation to meal duration, and (5) correcting data points deviating from trends using machine learning models. The custom computational code was written in SAS (9.4). For meal event (ME) and duration (MD) calculation, feeding events were summed continuously until the time gap between events did not exceed 20 min. If the time gap exceeded 20 min, the sum was reset, and the next event was considered a new meal. To ensure data reliability, only animal records with more than 80% consistent data were included in the analysis, resulting in the elimination of 10% of the animals. This left a final data set of 2,407 animals and over 10 million rows of data. The results indicate that the application of machine learning techniques—specifically, generalized additive models (GAM)—for modeling feed intake events yielded the most accurate outcomes and was extensively used in the computational framework. The algorithm produced the following results from final data set: an average of 77 ± 0.0951 feeding events and 8 ± 0.0058 meals per animal per day, with a daily feed intake of 11.87 ± 0.0075 kg per animal. The mean meal duration was 2 h and 13 min, and the average interval between meals was 2 h and 39 min.

Key Words: electronic feeder, data mining, machine learning

2142 Inhibition of methanogenesis with biochar in combination with nitrate in continuous culture of ruminal microbes. A. Kolganova^{*1}, J. L. Firkins¹, K. E. Mitchell³, B. Wenner⁴, and M. Minnema², ¹College of Food, Agricultural, and Environmental Sciences, The Ohio State University, Columbus, OH, ²Oregon Biochar Solutions, White City, OR, ³Elanco, Columbus, OH, ⁴FeedworksUSA, Cincinnati, OH.

Biochar (BC) has many redox-active chemical groups. We hypothesized that the potential for added NO_3^- to compete for aqueous H_2 [$\text{H}_2(\text{aq})$] to suppress methanogenesis would be enhanced by NO_3^- embedding in the BC matrix. Rumen fluid was collected from 2 cannulated Holstein cows to inoculate 5 dual-flow continuous cultures in 5 periods in a 5×5 Latin square design. All fermenters were fed 50 g (DM) of a pelleted 50:50 forage:concentrate diet every 12 h. The 5 treatments were control, BC (2% DM), NO_3^- (1.5% DM), BC + NO_3^- (2% DM both), and BC + NO_3^- (1% DM both). Buffer was infused at a rate of 10%/h with both filtrate outflow and solid outflow rates set to 5%/h. Periods were 8 d of adaptation followed by 4 d of sampling. Fermenter CH_4 and H_2 emissions, and $\text{H}_2(\text{aq})$ concentrations were measured using the

Micro-Oxymax detection system. Data were analyzed using protected least significant difference pairwise comparisons. Feeding BC and NO_3^- decreased ($P \leq 0.03$) daily CH_4 production by 36 and 45%, respectively. Combining BC and NO_3^- decreased ($P \leq 0.01$) daily CH_4 production by 71% and 89% for 2% and 1% doses, respectively, and by more ($P \leq 0.09$) than BC and NO_3^- alone. All treatments containing BC decreased daily H_2 emission ($P \leq 0.03$), but no significant difference in effects between treatments was observed ($P \geq 0.16$). Feeding 1.5% NO_3^- increased ($P = 0.06$) daily H_2 emission, which was decreased when BC was combined. Treatment interacted ($P = 0.02$) with time for $\text{H}_2(\text{aq})$. At 1 h post-feeding, NO_3^- and BC + NO_3^- (2%) increased ($P \leq 0.08$) $\text{H}_2(\text{aq})$ by 11.9 and 31.2 μM , respectively. At 6 h post-feeding, NO_3^- increased ($P \leq 0.01$) $\text{H}_2(\text{aq})$ by 17.9 μM . The 1% BC + NO_3^- combination decreased ($P \leq 0.08$) $\text{NH}_3\text{-N}$ concentration compared with all other treatments. The combination of BC and NO_3^- was most effective in CH_4 suppression, with BC potentially adsorbing H_2 or enhancing the potential of NO_3^- to divert $\text{H}_2(\text{aq})$ from methanogenesis.

Key Words: biochar, nitrate, methanogenesis

2143 Effects of productivity gain, allocation method, functional unit and quantification method on carbon footprint of milk produced under dual-purpose dairy production system in Bangladesh. M. S. Bari^{*1,2}, M. A. Islam², M. H. Rashid², and M. E. Uddin¹, ¹Department of Animal Science, University of Connecticut, Storrs, CT, ²Department of Dairy Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

Our objective was to determine the effects of productivity gain, functional units (FU), quantification and allocation methods of greenhouse gas on milk carbon footprint (CF) in dual-purpose dairy system in Bangladesh. An attributional cradle-to-farmgate life cycle assessment (LCA) was conducted following ISO-14040/14044 and FAO guidelines, incorporating feed production, enteric emissions, manure management, and farm operations in system boundary. Functional units compared were 1 kg fat- and protein-corrected milk (FPCM), 100 kcal energy, and 100 g protein. Foreground data from Bangladesh Agricultural University Dairy Farm (2018–2023) on herd structure, milk yield and composition, diets, manure management, and farm energy were collected via surveys, expert consultations, and literature. Enteric methane (CH_4), manure CH_4 , and nitrous oxide (N_2O) emissions were estimated using Intergovernmental Panel on Climate Change (IPCC) Tier 1 and Tier 2 methods or region-specific equations. Emissions were allocated between milk and meat using no allocation, biophysical, or economic methods when FPCM was used as FU. Over 5 years, milk yield was increased by 65%, leading to 23% to 40% reduction in CF (e.g., 8.33 vs. 5.03 kg $\text{CO}_2\text{-eq/kg}$ FPCM) depending on the FU and allocation methods used. The IPCC Tier 2 method reflects the production system better by using system-specific factors and had 18% lower CF than Tier 1 (e.g., 5.03 vs. 6.01 kg $\text{CO}_2\text{-eq/kg}$ FPCM). Similar to FPCM, the use of 100 kcal energy and 100 g protein as FU showed the same trend of reduction in CF due to gain in milk yield. Compared with no allocation (5.03 kg $\text{CO}_2\text{-eq/kg}$ FPCM), biophysical allocation reduced the CF at greater extent than economic allocation (e.g., 2.76 vs. 3.31 kg $\text{CO}_2\text{-eq/kg}$ FPCM) by putting less weight on milk (43%–60% vs. 54%–70%, depending on the production year). The major contributors to the CF were enteric fermentation (46%–49%), followed by manure management (29%–30%), feed production (15%–18%), and farm operations (6%–7%), highlighting the hotspots for mitigation strategies.

Key Words: carbon footprint, dual-purpose dairy system, allocation method

2144 Greenhouse gas emissions from animal and manure management: Evaluating COMET-Farm and RuFaS in 2 US dairies. H. Hu*, *Department of Animal Science, Cornell University, Ithaca, NY.*

This study compares the greenhouse gas (GHG) estimation capabilities of COMET-Farm, a free web-based tool, and the Ruminant Farm Systems (RuFaS) model, an emerging on-farm simulation tool. Our objective is to provide benchmarks that help users determine which tool best meets their needs. We evaluated GHG emissions from animal and manure management on 2 US Holstein dairy farms (Farms A and B) with 1,002 and 940 cows. Each farm was simulated with RuFaS (v0.9.2) over 4 iterations, and the results were averaged. RuFaS estimates were converted to metric tonnes of CO₂-equivalent (t of CO₂-eq) per year using global warming potentials of 28 for CH₄ and 265 for N₂O with no emission allocation applied, matching COMET-Farm outputs. Due to missing farm-specific data, we supplemented COMET-Farm (v5.0) inputs with additional RuFaS outputs, such as average daily dry matter intake, dietary nutrient composition, average body weight, and manure storage surface area. Calves were excluded due to their minimal emissions and omission from COMET-Farm. Both tools produced almost identical estimates for lactating cow enteric CH₄ emissions: for Farm A, COMET-Farm and RuFaS estimated 3,516.5 and 3,528.5 t of CO₂-eq/year, respectively; for Farm B, 3,413.8 and 3,425.2 t of CO₂-eq/year. This close agreement is expected, as both tools use the same prediction function. For manure management involving anaerobic lagoon with cap and flare (Farm A's close-up and lactating animals), COMET-Farm estimated zero storage CH₄ emissions, using a methane conversion factor of 0, and RuFaS, accounting for CH₄ leakage during combustion, predicted 68.9 t of CO₂-eq/year for close-up animal manure and 646.9 t of CO₂-eq/year for lactating cow manure. Similarly, COMET-Farm estimated zero direct manure storage N₂O emissions, whereas RuFaS predicted 14.3 t of CO₂-eq/year for close-up animal manure and 109.9 t of CO₂-eq/year for lactating cow manure, using an emission factor of 0.005. These findings offer a reference for comparing GHG estimates from different tools and show that RuFaS can deliver farm-specific estimates. Future work will extend this comparison to a broader range of farms and practices.

Key Words: greenhouse gas, COMET-Farm, RuFaS

2237 Effect of milking frequency before dry-off on milk yield and mammary health in dairy cows. J. Gao*, C. G. Savegnago, N. L. P. Kant, A. M. Roper, T. N. Marins, and S. Tao, *Department of Animal and Dairy Science, University of Georgia, Athens, GA.*

Dry-off (DO) is challenging for high-producing cows and reducing milking frequency (RMF) is a practice to lower milk yield before DO. We aimed to study the effects of RMF on milk yield and mammary health in the next lactation in a commercial dairy farm. During lactation, cows were milked 3× per day. At -13 d relative to DO (DRD), cows were assigned to 2 groups based on the last digit number (even/odd) of her name tag: milked 3× per day until DO (n = 480) or 2× per day (n = 516) by skipping the last milking of a day. The 3× and 2× cows had similar ($P \geq 0.32$) parity and milk yield before the onset of treatments and housed in adjacent pens under the same barn. After the last milking, all cows received intramammary antibiotics and teat sealants. After DO, all cows were housed together in barns and managed similarly. Pooled milk samples were collected within 3 d postcalving from each cow and cultured to detect common mastitis pathogens. Blood samples were drawn at d -14, 1 (the day of DO, immediately after the last milking), 2 and 9 DRD to assess plasma haptoglobin (HP), cortisol and hematological profiles. Mastitis incidence up to 280 DIM in the next lactation was

recorded. Data were analyzed using mixed model and logistic regression. Reducing milking frequency decreased ($P < 0.01$) MY before DO, with the greatest decline (48%) on d -13 DRD; however, the difference in MY reduced to 11% on the day of DO. Milking 2× per day increased ($P < 0.01$) circulating neutrophil counts on d 1 DRD compared with 3×. Regardless of treatment, circulating monocytes, lymphocytes, and total white blood cell counts decreased on d 2 DRD compared with pre-DO levels ($P < 0.01$). Treatments did not affect circulating HP and cortisol concentrations ($P \geq 0.60$). RMF did not affect ($P = 0.92$) culture positive incidence in primiparous cows but increased positive culture incidence (12% vs. 7%, $P = 0.03$) in multiparous cows. Treatments did not affect mastitis incidence in either primiparous cows ($P = 0.32$) or multiparous cows ($P = 0.24$) in the next lactation. In conclusion, RMF for 13 d before DO decreased MY but increased culture positive incidence in early lactation.

Key Words: dry-off, milking frequency, mammary health

2238 Effect of red seaweed on enteric methane emissions and performance of dairy cows: A meta-analysis. M. A. Rahman*, M. R. A. Redoy², P. R. Sristi³, M. Uddin⁴, T. Sultana⁵, N. R. Das³, S. Z. Seema⁴, K. A. J. Esha⁴, A. Irawan⁶, and W. M. Sims¹, ¹*Department of Agriculture, Nutrition and Food System, University of New Hampshire, Durham, NH,* ²*Department of Animal Science, University of Connecticut, Storrs, CT,* ³*Department of Animal Science, Bangladesh Agricultural University, Mymensingh, Bangladesh,* ⁴*Department of Animal Science and Nutrition, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh,* ⁵*Department of Dairy Science, Bangladesh Agricultural University, Mymensingh, Bangladesh,* ⁶*Vocational School, Universitas Sebelas Maret, Surakarta, Indonesia.*

This meta-analysis aimed to evaluate the effects of red seaweed (RSW) on methane (CH₄) emissions, performance, rumen fermentation, and digestibility in dairy cows. A systematic literature search yielded 17 peer-reviewed studies for final meta-analysis. Data were analyzed using a mixed effect model, with treatment as a fixed effect and study variability as random effect. Weighted mean differences (WMD) were calculated using inverse variance weighting, with 95% CI estimated from study-level variances. Segmented meta-regression analysis was conducted to evaluate the dose-response relationship between daily bromoform (CHBr₃; mg/d) intake and CH₄ reduction. Our results indicated that RSW reduced daily CH₄ by 26.6% compared with the control (-135.3 g/d; $P < 0.01$), alongside reductions in CH₄ yield (CH₄/DMI; -21.8%, $P < 0.01$) and intensity (CH₄/MY; -15.9%, $P < 0.01$). Furthermore, subgroup analysis suggested that *Asparagopsis taxiformis* tended to reduce CH₄ more than *Asparagopsis armata* (95% CI: -175.6 to -132.8 g/d vs. 95% CI: -159.3 to -101.7 g/d), though the difference was not statistically significant ($P = 0.09$). In contrast, CHBr₃ intake exhibited a nonlinear dose response with daily CH₄. A steep decline in daily CH₄ was observed between 0 and 319.53 mg/d CHBr₃ intake ($\beta = 200.03$, 95% CI: 134.74 to 265.31, $P < 0.01$), followed by a weaker trend beyond this threshold ($\beta = 77.85$, 95% CI: -16.05 to 171.75, $P = 0.01$). However, high heterogeneity ($I^2 = 94.7\%$) indicated a variable responses across studies, warranting a bigger data set to improve the precision of breakpoint regression. Furthermore, RSW decreased DMI by 1.91 kg/d ($P < 0.01$), leading to a reduction in milk yield (-1.19 kg/d; $P < 0.01$) and ECM (-1.33 kg/d; $P < 0.01$). In contrast, NDF digestibility was not affected by RSW ($P = 0.27$), indicating no impairment in fiber utilization. Furthermore, RSW increased the relative proportion of rumen propionate (+6%, $P < 0.01$) and butyrate (+11.6%, $P < 0.01$), but reduced acetate (-6.25%, $P < 0.01$) and total VFA (-10.98 mmol/L; $P < 0.01$) with $I^2 > 95\%$, suggesting substantially variability among study

conditions. Overall, RSW are promising to reduce CH₄ emissions in dairy cows, but more effective feeding strategies or doses are required to avoid potential negative effects on milk yield and DMI.

Key Words: *Asparagopsis taxiformis*, feed additive, livestock

Ruminant Nutrition 1: Calves and Heifers—Posters

2145 Stability of a *Bacillus*-based direct-fed microbial in whole milk and milk replacer. O. C. M. Queiroz*, C. Galschiet, S. Thysen, M. Schjelde, A. Segura, and B. I. Cappelozza, *Novonesis, Lyngby, Zealand, Denmark*.

In dairy farms, producers often offer bacteria-based direct-fed microbials (DFM) to pre-weaning calves through whole milk or milk replacers. However, to our knowledge, few studies have evaluated the stability of bacteria-based DFM post-addition to whole milk and milk replacer for up to 4 h. Hence, this study evaluated the stability of a *Bacillus*-based DFM in whole milk and milk replacer. Three independent fermentation batches of a DFM containing *Bacillus licheniformis* 809 and *Bacillus subtilis* 810 (Bovacillus; Novonesis, Lyngby, Denmark; BAC) were used in these experiments. For each replicate at h 0, 10 g of BAC was suspended in 90 mL of tap water. The initial dilution was treated in a stomacher for 2 min to obtain a homogeneous suspension and the resulting suspension was diluted into whole milk and milk replacer at 37°C to a target final concentration $\geq 7.0 \times 10^5$ cfu/mL. Additional 100-mL aliquots were stored at 25°C for 1 and 4 h. The *Bacillus* spores were counted in the laboratory and within each independent batch, samples were run in duplicates. All data were analyzed using the MIXED procedure of SAS, using the flask as the experimental unit, whereas batch and hour were added in the fixed effects. No hour $\times$ batch interactions were detected herein ($P \geq 0.41$). No main hour effect was observed in the recovery of the *Bacillus* spores in whole milk ($P = 0.89$), indicating the stability of this bacteria-based DFM (Table 1). The concentration of *Bacillus* spores in the milk replacer was constant until 4 h, when a slight increase was observed in the *Bacillus* spp. counts ($P = 0.05$). In summary, the *Bacillus*-based DFM remained stable in whole milk and milk replacer for up to 4 h after mixing.

Key Words: *Bacillus*-based direct-fed microbial, milk, stability

2146 Combinations of different liquid and solid diets and behavior of dairy calves around weaning. C. M. M. Bittar*, I. C. R. Oliveira¹, J. M. Feliciano¹, I. Vitoria², G. E. Scheineider³, L. A. R. Silva¹, J. S. Silva¹, J. M. Freire¹, E. W. Carneiro⁴, and C. Hilgert⁴, ¹Department of Animal Sciences, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, Sao Paulo, Brazil, ²Instituto Federal Sudeste de Minas Gerais, Rio Pomba, Minas Gerais, Brazil, ³Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil, ⁴Agrifirm Brasil, Curitiba, Paraná, Brazil.

Table 1 (Abstr. 2145). Counts of the *Bacillus* spores in whole milk and milk replacer

Item	BAC counts, cfu/mL	SEM	Overall P-value	P-value vs. h 0
Whole milk			0.89	
h 0	9.22E+05	1.85E+04		—
h 1	9.15E+05	1.85E+04		0.80
h 4	9.28E+05	1.85E+04		0.80
Milk replacer			0.08	
h 0	8.40E+05	2.12E+04		—
h 1	8.63E+05	2.12E+04		0.15
h 4	8.93E+05	2.12E+04		0.05

The balance between the liquid diet (LD) and solid diet (SD) intake is crucial for successful weaning. Feeding a more complex solid diet, such as a textured (Text) compared with a micropellet (Micro) starter, as well different LD composition, may affect performance and animal behavior, especially around weaning. The objective of this study was to evaluate the association of LD alternatives with different SD and their effects on calf behavior during preweaning and immediate postweaning periods. Sixty Holstein calves were used in a 2×2 factorial design, with 2 sources of LD (milk replacer [22% CP and 17% fat] diluted to 15% solids [MR] or powdered whole milk [24.5% CP and 26.5% fat] diluted to 12.5% solids and added with 25 g/L of milk replacer to achieve 15% solids [WM+]); and 2 types of SD (Micro or Text), both with 22% CP and 10% ADF. Calves were individually housed and fed 8 L/d until wk 3 and then 6 L/d of the respective LD until 56 d of age, when gradual weaning began by reducing volume by 1 L/d, and calves were completely weaned at 63 d. Calves had free access to water and the respective concentrate. Hay was fed ad libitum from the seventh week. Data were analyzed using the MIXED procedure of SAS, with treatments as fixed effect, and the means were compared using Tukey test. Orthogonal contrasts were built to test the effect of LD and SD. Behavior was evaluated by an instant collection with focal sampling with 5-min intervals at wk 7, 8, and 9 of age for 10 h (7 a.m. to 5 p.m.) between the LD feeding schedule. Except time drinking water, ruminating, liking housing, non-nutritive suckling, and urinating+defecating, all the other behaviors were affected by age of the calves ($P < 0.02$). There was an increasing time standing, eating concentrate, grazing, and vocalizing, but a decreasing time laying down, self-grooming, and exploring the environment as calves aged. The combination of LD and SD did not affect calves behaviors, however feeding MR increased time laying down but not sleeping ($P < 0.05$). In addition, feeding Text increased time ruminating ($P < 0.03$) and tended to increase time eating concentrate ($P < 0.07$). Age was more effective in changing behavior; however, the more complex starter improved time eating and ruminating, which may influence performance during the transition period.

Key Words: micropellet, eating time, rumination time

2147 Performance and health of calves fed calf starters varying in physical form during the preweaning and early postweaning period. E. Dufour*, A. Plumski², B. Ziegler¹, A. Golombeski¹, and I. Salfer³, ¹Hubbard Feeds, Mankato, MN, ²University of Minnesota Southern Research and Outreach Center, Waseca, MN, ³University of Minnesota, Saint Paul, MN.

Calf starters (CS) have varied in presentation since the commercialization of the US dairy industry, with meal CS seeing a resurgence in popularity due to the economic benefit they can offer. The objective of this study was to evaluate performance and health of calves fed pelleted, texturized, or meal mix CS. Holstein heifer calves ($n = 108$; 40.8 ± 0.73 kg) were assigned to 1 of 4 treatments in a randomized complete block design for 49 d preweaning and 7 d postweaning. Treatments included CS fed ad libitum throughout the study, formulated as 1) complete pellet (PEL); 2) texturized (TEX); 3) meal with steam flaked corn (M-SF); and 4) meal with fine ground corn (M-FG). Crude protein and starch of all CS were as follows, respectively (% DM): 1) 20.0%, 24.1%; 2) 21.7%, 29.0%; 3) 21.5%, 26.0%; and 4) 21.5%, 28.2%. Calves received 283.5 g of 24% CP, 20% fat milk replacer (MR) 2 $\times$ /d from d 1 to 7, 340.1 g 2 $\times$ /d from d 8 to 42, and 1 $\times$ /d from d 43 to 49. Body weight was measured at d 0, 14, 28, 42, 49, 56, and 84. Hip height (HH) was measured

at d 1, 56, and 84. Fecal scores were measured weekly. Feed intake was measured daily and calculated bi-weekly. Data were analyzed as a linear mixed model in R with fixed effect of treatment, and random effects of BW, source herd and location. No effect of MR intake, HH, or scouring frequency was observed throughout the study. During the post-weaning period (d 50–56), ADG of M-SFC (1.24 kg/d) was significantly higher ($P = 0.02$) than PEL (1.03 kg/d), with TEX and M-FG ADG being intermediate at 1.12 and 1.18 kg/d, respectively. Postweaning CS intake was significantly higher ($P = 0.02$) for M-SF (2.47 kg/d), and M-FG (2.47 kg/d) compared with PEL (2.16 kg/d), with TEX being intermediate (2.3 kg/d). Overall, only slight differences occurred in the postweaning phase of this study, suggesting the different physical forms of CS presented in this study can be viable options for raising pre and post weaned calves when these diets are properly balanced. Further research is needed to determine transition success and long-term performance of the various CS forms used in this study.

Key Words: calf nutrition, meal feed

2148 Performance and health of postweaning Holstein heifers offered a low-moisture-block self-fed nutritional supplement with varying levels of menthol. E. Dufour^{*1}, A. Plumski², M. Robbins³, J. Peine³, B. Ziegler¹, A. Golombeski¹, and I. Salfer⁴, ¹Hubbard Feeds, Mankato, MN, ²University of Minnesota Southern Research and Outreach Center, Waseca, MN, ³Ridley Block Operations, Mankato, MN, ⁴University of Minnesota, Saint Paul, MN.

Newly weaned heifers are susceptible to low feed intake and respiratory disease due to the stress of diet changes, pen and group changes, and management changes that often accompany the postweaning phase. With concern of antibiotic resistance in animal agriculture, recent research has focused on the efficacy of feeding natural products, such as essential oils and their active compounds, to prevent and treat illness in livestock species. Menthol (MTL) is a naturally occurring compound shown to assist in easing respiratory issues by reducing mucous production. The objective of this study was to determine the effect of low moisture blocks (LMB) formulated with varying rates of MTHL on health, performance, and intake of dairy heifers 2 to 4 mo of age (BW = 83.8 ± 1.79 kg on d 1). Sixteen pens ($n = 4$ /treatment) of 7 post-weaned heifers were randomly assigned to 1 of 4 LMB treatments with MTL supplemented at the following rates: 1) 0.5 g/kg (M1); 2) 1.0 g/kg (M2); 3) 1.5 g/kg (M3); and 4) 2.0 g/kg (M4). Heifers were on treatments for 56 d. Feed intake was measured daily and BW was measured on d 1, 28, and 56. All data were analyzed as a generalized linear model in R with fixed effect of treatment and initial BW used as a covariate for BW, BW gain, and intake. No differences were observed for grain, hay, or LMB intake, or feed efficiency ($P > 0.10$). There was a tendency ($P = 0.07$) for increased BW for M3 (118.0 kg) over M1 (110.2) at d 28, with M2 and M4 being intermediate at 115.6 kg and 112.7 kg, respectively. Hip height tended to be greater ($P = 0.06$) for M2 (101.8 cm) and M3 (101.6 cm) compared with M1 (99.6 cm), with M4 being intermediate at 101.3 cm. Although not statistically significant, M2 did not necessitate treatment for respiratory disease, whereas M1, M3 and M4 groups received treatment after showing clinical signs of respiratory disease over the course of the 56 d study. The results of this study suggest that MTL supplementation of 1.0 to 1.5 g/kg in a LMB can improve performance and may improve overall respiratory health of heifers from 2 to 4 mo of age.

Key Words: low moisture block, menthol, respiratory

2149 Encapsulated magnesium butyrate influences dairy cow colostrum composition and may facilitate IgG absorption in the

neonatal calf. A. Bach^{*1,2} and J. Edwards³, ¹Department of Animal Science, University of Lleida, Lleida, Spain, ²ICREA, Barcelona, Spain, ³Palital Feed Additives, Veldriel, the Netherlands.

The aim of this study was to evaluate the effect of encapsulated magnesium butyrate (MgB) on colostrum and IgG absorption in the neonatal calf. Fifty-five days before expected calving, 167 multiparous Holstein cows were blocked by calving date and randomly allocated to 3 groups. Each group received the same TMR (1.20 Mcal of NEL/kg, 54.5% NDF, and 13.0% CP) until calving with MgB (Rumen-Ready, Palital Feed Additives, the Netherlands) supplemented as follows: 0 g/cow per day ($n = 59$), 100 g/cow per day ($n = 54$), and 150 g/cow per day ($n = 54$). Colostrum volume and composition were measured. Each calf ($n = 141$) was fed 4 L of colostrum (from a cow on the same treatment as its dam) and colostrum intake recorded. Calf birth weight was measured, and at 24 h, serum concentrations of IgG, insulin, and glucose were determined. Data were analyzed using ANOVA and linear regression analyses. Calf birth weight was not affected by MgB (40.9 ± 0.80 kg). In contrast, MgB did affect colostrum concentrations of IgG, insulin and fat, and insulin intake (Table 1). Serum IgG ($r = 0.41$) as well as apparent efficiency of absorption (AEA) of IgG ($r = 0.28$) were positively correlated with insulin intake. There was a tendency for serum insulin ($r = 0.17$) and glucose ($r = 0.17$) concentrations to be positively correlated with insulin intake. In conclusion, MgB supplementation of dry cows influences colostrum composition, resulting in increased insulin intake of calves which may facilitate IgG absorption.

Key Words: dry period, insulin, immunity

2150 Effects of medium-chain fatty acid source in milk replacer on dairy calf performance and health for 7 weeks preweaning and 1 week postweaning. A. Plumski¹, D. Ziegler², W. P. Hansen², M. F. Scott^{*2}, and I. Salfer³, ¹University of Minnesota Southern Research and Outreach Center, Waseca, MN, ²Actus Nutrition, Eden Prairie, MN, ³University of Minnesota, Saint Paul, MN.

The objective of this experiment was to determine the effects of 2 different sources of medium chain fatty acids (MCFA) when added to the protein encapsulated fat (PEF) in milk replacer on the health and performance of Holstein dairy calves for 7 wk preweaning and 1 wk postweaning. Holstein calves ($n = 116$; 2 to 5 d of age) were assigned to 1 of 5 treatments in a randomized complete block design ($n = 21$ to

Table 1 (Abstr. 2149). Effect of magnesium butyrate (MgB) on colostrum and calf-related parameters

Parameter	MgB (g/cow per day)			SE	P-value
	0	100	150		
Colostrum yield, kg	4.8	5.1	5.5	0.43	0.62
Colostrum IgG, mg/mL	121.0 ^a	101.2 ^b	108.3 ^{ab}	5.39	0.03
Colostrum IgG yield, g	577	513	590	47.3	0.49
Colostrum insulin, ng/mL	106 ^b	165 ^a	150 ^a	0.08	<0.01
Colostrum fat, %	6.61 ^b	7.51 ^a	7.04 ^{ab}	0.22	<0.01
Colostrum protein, %	15.2	14.6	15.0	0.19	0.19
Colostrum intake, L	3.66	3.82	3.51	0.12	0.12
IgG intake, g	443	388	379	25.21	0.08
Insulin intake, μ g	4.09 ^a	6.39 ^b	5.20 ^{ab}	0.47	<0.01
Serum IgG, mg/mL	29.5	29.1	30.0	2.08	0.95
Serum insulin ng/mL	0.55	0.76	0.57	0.91	0.24
AEA of IgG, %	27.7	30.9	30.3	1.79	0.31

^{a,b}Values with uncommon superscripts differ at $P < 0.05$.

25/treatment). Treatments included milk replacer (24% CP, 20% fat) with PEF containing 50% lard-tallow fat (LTF) and 50% palm oil ([PO]; CON); 45% LTF, 45% PO and 10% coconut oil ([CO]; CO10), 45% LTF + 45% PO + 10% palm kernel oil ([PKO]; PKO10), 38% LTF + 38% PO + 24% CO (CO24), or 38% LTF + 38% PO + 24% PKO (PKO24). Calves were fed 340.2 g MR/d (12.5% wt/vol in water) 2×/d from d 1 to 42, and 1×/d from d 43 to 49 and offered water and calf starter (CS; Elite 18 Calf Starter, Hubbard Feeds) ad libitum. Body weight was measured on d 1, 14, 28, 42, 49, and 56. Hip height (HH) was recorded on d 1, 49, and 56. Intake of MR and CS were calculated daily and summarized biweekly. Fecal scores were evaluated on 1 to 4 scale (1 = normal; 2 = loose, pudding; 3 = very loose, no watery separation; and 4 = very watery) daily. Data were analyzed as a 2 × 3 factorial with a linear mixed model testing the main effects of MCFA source, MCFA concentration and their interaction, as well as the random effects of source herd and nursery room. From d 1 to 14, there was an interaction between MCFA source and concentration on ADG with CON (0.39 kg/d), CO10 (0.40) and PKO24 (0.39) having greater ($P = 0.05$) ADG than PKO10 (0.36) and CO24 (0.36). Furthermore, average fecal score from d 15 to 28 was greatest in PKO10 (1.49) and CO24 (1.49), and lowest in CO10 (1.31), with intermediate values for CON (1.40) and PKO24 (1.41). There were no differences in HH gain ($P > 0.68$) or milk replacer or starter intake ($P > 0.16$) in either the preweaning period or entire experiment. The response of MCFA concentration in MR of calf performance and health appears to be dependent on MCFA source.

Key Words: calf nutrition, medium-chain fatty acid, average daily gain

2151 Health and growth of preweaning Holstein cross calves fed varying amounts of B vitamins in milk replacer. K. N. Brost^{*1}, M. L. Pister¹, A. Saito², and J. K. Drackley¹, ¹University of Illinois Urbana-Champaign, Urbana, IL, ²The National Federation of Dairy Co-operative Associations (Zen-Raku-Ren), Tokyo, Japan.

The aims of this study were to determine the effects of B vitamin concentrations on health and growth of preweaned calves through 8 wk of age. The trial was a randomized complete block design with 48 Holstein-Angus cross calves (28 male, 20 female), blocked by BW and serum total proteins. Calves were transported from a local dairy at ≤4 d of age. Milk replacer (MR; 28% CP, 15% fat) was fed in a step-up step-down protocol: 600 g/d for d 0–7 and 50–56, 800 g/d for d 8–14 and 43–49, and 1,125 g/d for d 15–42. Water and starter were offered ad libitum from d 0 and d 43, respectively. The B vitamins of focus were B1, B3, B6, and B12. Treatments were basal MR with no added B vitamins (CON); MR with B vitamins above basal (BP; 4.27 mg/kg of B1, 3.86 mg/kg of B3, 0.54 mg/kg of B6, and 18 µm/kg of B12); and MR with B vitamins above the 2021 NASEM recommendations (NP; 28.8 mg/kg of B1, 18.6 mg/kg of B3, 9.17 mg/kg of B6, and 254 µm/kg of B12). Custom powered blends were added to MR before packing. Health scores (scale of 1–4) and MR intake were recorded twice daily, and starter intake was recorded daily. Frame growth and BW were recorded weekly. Electrolytes (ECT) were offered as needed for scours and pyrexia. Health data were analyzed with GLIMMIX, and growth and intake data used the MIXED procedure in SAS. Initial and final BW did not differ among groups. Growth for wk 1–8 revealed BP had greater ($P = 0.003$) BW compared with CON. Similarly, ADG was greater ($P = 0.03$) for BP relative to CON. Hip height was greater ($P = 0.02$) for BP and NP compared with CON. Withers height tended to be greater ($P = 0.07$) for BP, and body length for NP was less ($P = 0.05$) both compared with CON. Weeks 7–8 starter intake was greater ($P = 0.03$) for NP relative to CON. For wk 1–8, days with ocular scores >1 were greater ($P = 0.05$) for NP in relation to CON. During wk 1–3 the

amount of ECT consumed (L) was less ($P = 0.02$) for BP and tended to be less ($P = 0.06$) for NP compared with CON. Greater growth was noted for BP, and NP to a lesser extent. This, combined with greater starter DMI, may suggest improved calf well-being and digestive tract maturity with added B vitamins. These results should be viewed with caution because final BW did not differ, and few health benefits were observed.

Key Words: calf, B vitamin, performance

2152 Fecal dry matter in Holstein bull calves fed high-solids milk replacers. M. L. Pister^{*} and J. K. Drackley, University of Illinois at Urbana-Champaign, Urbana, IL.

We aimed to assess whether fecal DM percentage (FDM%) was linked to fecal score and influenced by nutrition. Holstein calves were fed milk replacer (MR) with low (13%) or high (17% or 18%) solids concentrations with different nutritional additives in Latin square designs. Fecal scores were recorded twice daily using a 4-point scale. Approximately 50 g of feces were collected daily from each calf via rectal palpation during sampling periods. Data were analyzed as Latin squares using MIXED and GLIMMIX procedures in SAS. Experiment 0 included 8 calves assigned to 1 of 4 treatments: low concentration and low DMI (CON), low concentration and high DMI (LH), high concentration and low DMI (HL), or high concentration and high DMI (HH). We determined a difference in FDM% between scouring (17%) and non-scouring (22%) calves ($P < 0.001$). Fecal DM% tended to be greater for CON compared with LH ($P = 0.06$). In experiment 1, 10 calves were assigned to 1 of 5 treatments: low solids % MR, high solids % MR, high solids with NaCl, high solids with psyllium, or high solids with casein. Although there was a difference in FDM% in scouring (22%) and non-scouring (24%) calves ($P < 0.001$), DM% did not differ among treatments. In experiment 2, 10 calves were assigned to 1 of 5 treatments: low solids % MR (PCON2), high solids % MR (NCON2), high solids with mineral mix (MM), high solids with psyllium (P2), or high solids with guar gum (GG). Fecal DM% was different between scouring (15%) and non-scouring (19%) calves ($P < 0.001$). Fecal DM% was greater for PCON2 compared with NCON2 ($P = 0.05$). A numerical improvement of 3 to 5 percentage units was observed in FDM% of MM, P2, and GG compared with NCON2. In experiment 3, 21 calves were assigned to 1 of 5 treatments: low solids % MR, high solids % MR, high solids with casein, high solids with psyllium, or high solids with casein plus psyllium. Although FDM% differed between scouring (14%) and non-scouring (19%) calves ($P < 0.001$), no difference was found among treatments. Fecal scores correctly classified degree of scouring as confirmed by FDM%. Further research is needed to determine the potential to use additives to ameliorate FDM% with a higher solids diet.

Key Words: calf, feces, dry matter

2153 Effects and mechanisms of *Schizochytrium* supplementation on *Escherichia coli* K99-induced calf diarrhea. Z. L. Fu^{1,2}, L. Ma¹, N. Malmuthuge³, L. L. Guan^{2,4}, and D. P. Bu^{*1}, ¹Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, ²University of Alberta, Edmonton, AB, Canada, ³Agriculture and Agri-Food Canada, Lethbridge, AB, Canada, ⁴University of British Columbia, Vancouver, BC, Canada.

Neonatal calf diarrhea is a major challenge in the dairy industry, with limited effective solutions. The microalgae *Schizochytrium* spp. (SZ), rich in n-3 fatty acids (FA), shows potential in preventing calf diarrhea, but its efficacy and mechanisms are unclear. This study evaluated preventive and therapeutic effects of SZ on *Escherichia coli* K99-induced diar-

rhea and its antioxidant capacity in neonatal calves. Sixty-five newborn calves were randomly assigned to one of 5 groups: control (CON), K99 challenged only (K99), antibiotic treatment (AB), SZ-prevention (SZP), and SZ-treatment (SZT). Except for CON, all calves were challenged with *E. coli* K99 on d 21 of age (d 21). Group SZP received SZ from d 7 to 20, whereas SZT received SZ from d 22 to 35. The AB group received gentamicin for 3 d after challenge once diarrhea occurred. Fecal scores were recorded daily to calculate diarrhea rate (diarrhea days/total days). Serum and fecal samples were collected on d 7, 21, 22, 25, 28, and 35 to measure oxidative stress markers (ROS, MDA, H₂O₂, antioxidant enzymes). Data were analyzed using a repeated measurement model in R (nlme, v4.1.0), with treatment and time as fixed effects and calf as a random effect. Before d 21, diarrhea rate in SZP was lower than in other groups (29% vs. 47%, $P < 0.05$). After d 21, the rate was higher in K99 than in CON (64% vs. 19%, $P < 0.05$), but was lower in AB and SZP than in K99 (49% and 27% vs. 58%, $P < 0.05$). The rate in SZT was similar with K99 on d 21 to 28 (63% vs. 58%) but was lower on d 29 to 35 (19% vs. 58%, $P < 0.05$). Oxidative stress analysis revealed that serum superoxide dismutase (SOD) activity was higher in SZP than in other groups on d 7 to 21 ($P < 0.05$), indicating enhanced antioxidant ability. After d 21, SOD activity was higher and ROS levels lower in SZP and SZT than in AB and K99 ($P < 0.05$). These effects align with prior studies supplementing n-3 FA to calves, which improved calf health and antioxidant enzyme activity of calves. However, SZ may show additional benefits through other bioactive compounds. The preventive and therapeutic effects of SZ operate through different mechanisms and require further investigation.

Key Words: calf diarrhea, *Schizochytrium* spp., antioxidant capacity

2154 Performance and health of calves fed milk replacer with or without a mannan oligosaccharide and bacillus additive. E. Dufour^{*1}, A. Plumski², D. Graugnard³, B. Ziegler¹, A. Golombeski¹, and I. Salfer⁴, ¹Hubbard Feeds, Mankato, MN, ²University of Minnesota Southern Research and Outreach Center, Waseca, MN, ³Alltech, Nicholasville, KY, ⁴University of Minnesota, Saint Paul, MN.

Efficient nutrient utilization in dairy calves is vital for optimal immune system function in early life and long-term performance of the animal. Improving feed efficiency (G:F) in growing calves can be accomplished by feeding a balanced calf starter (CS) and milk replacer (MR) diet, and by the use of feed additives designed to optimize overall nutrient utilization. Mannan oligosaccharides (MOS) are research-proven additives designed to improve gut health and overall nutrient processing in calves, but limited research has been done on feeding MOS alongside strains of *Bacillus* (BAC) bacteria, which may further improve performance in dairy calves. The objective of this study was to determine performance and health of calves fed MR supplemented with or without a MOS and BAC blend. Holstein heifer calves (n = 106) were assigned to 1 of 2 treatment groups for 49 d preweaning and 7 d postweaning. Treatments were as follows: 1) a control 24% CP, 20% fat MR fed at 340.1 g 2×/d from d 1 to 42, and 1× from d 43 to 49 (CON); and 2) the control MR supplemented with MOS and BAC at 1.6 g/d (BMB). All calves were fed 18% CP CS and water ad libitum throughout the study. Calves were housed individually in curtain-sided barns. Body weight was measured at d 0, 14, 28, 42, 49, and 56. Fecal scores were measured weekly. Feed intake was measured daily and calculated bi-weekly. Data were analyzed as a linear mixed model in R with fixed effect of treatment, and random effects of BW, source herd and location. No differences on MR or CS intake, hip height gain, or health events were observed throughout the study. Postweaning ADG tended to be greater ($P = 0.09$) for BMB (1.03 kg/d) over CON (0.93 kg/d). This translated to greater G:F in the

postweaning phase for BMB over CON at 0.44 and 0.39 kg/kg ($P = 0.02$), and an increase in G:F for BMB over CON at 0.51 and 0.50 kg/kg over the entire 56 d study ($P = 0.04$), respectively. Overall, results of this study demonstrate that supplementing calves with a MOS and BAC blend in MR improved G:F and nutrient utilization throughout the milk feeding phase and immediately postweaning.

Key Words: dairy calf, mannan oligosaccharide, *Bacillus*

2155 Effects of increased concentrations of dried cheese byproduct in milk replacer on growth, feed intake, and scouring frequency of dairy calves for 7 weeks preweaning and 1 week postweaning. A. Plumski¹, D. Ziegler², B. Hansen², M. Scott², and I. Salfer^{*3}, ¹University of Minnesota Southern Research and Outreach Center, Waseca, MN, ²Actus Nutrition, Eden Prairie, MN, ³University of Minnesota, Saint Paul, MN.

Dried cheese (DC) byproduct is an ingredient from the food industry and can be used to provide milk-based fat and protein for calves. We have previously observed no adverse effects of DC on calf performance. The objective of this study was to revisit the effects of titrated levels of dried cheese byproduct in the milk replacer (MR) on the health and performance of Holstein dairy calves for 7 wk preweaning and 1 wk postweaning. Holstein calves (n = 104; 2 to 5 d of age) were assigned to 1 of 4 treatments in a randomized block design, including: control milk replacer (24% CP [whey] 20% fat [tallow, lard, cheese, coconut]) containing no DC (DC0), or DC replacing whey protein at 4.38% (DC4), 8.75 (DC9), or 17.5% (DC18) of MR (n = 26/treatment). Calves were fed 340.2 g MR/d (12.5% wt/vol in water) 2×/d from d 1 to 42, and 1×/d from d 43 to 49, and offered water and calf starter (CS) ad libitum. Body weight was measured on d 1, 14, 28, 42, 49, and 56 and ADG was calculated. Hip height (HH) was recorded on d 1, 49, and 56. Intake of MR and CS were calculated daily and summarized biweekly. Fecal scores were recorded daily. Data were analyzed as a linear mixed model with fixed effect of treatment and random effects of source herd and nursery room, and linear and quadratic contrasts. ADG differed by treatment from d 29 to 42 ($P = 0.04$), being greater in DC9 than DC4 (0.81 vs. 0.66 kg/d, SE = 0.04; $P = 0.04$), but not different between from DC0 (0.70 kg/d) or DC18 (0.73 kg/d). Similarly, HH gain tended to be greater in DC9 than DC4 (10.6 vs. 9.28 cm, SE = 0.71; $P = 0.09$) from d 1 to 49, but not different from DC0 (9.58 cm) and DC18 (9.47 cm). Fecal score was linearly reduced from d 29 to 42 by increased DC concentration ($P = 0.002$). No differences in MR ($\mu = 599$ g/d; SE = 4.54; $P = 0.91$) or CS ($\mu = 658$ g/d; SE = 45.4; $P = 0.91$) intake were observed. Results suggest that replacement of whey protein with DC at 8.75% of MR can result in modest improvements in ADG and scouring in the preweaning period.

Key Words: calf nutrition, dried cheese, average daily gain

2156 Effects of different applications of oregano essential oil on performance and health of rearing calves. A. Beckmann* and T. Borchardt, Dostofarm GmbH, Westerstede, Germany.

During calf rearing, animals from different farms are brought together, leading to increased infection pressure, which can negatively affect both health and performance. The objective of this study was to evaluate the effects of oregano essential oil (OEO) supplementation in milk replacer (MR) and starter feed (SF) on the growth performance and health status of rearing calves. For the trial (June to August 2023), 321 male Holstein calves (age 14 to 20 d) from various farms were divided into 4 homogeneous groups (randomized block design): control group

(CON), treatment with 75 g/mt MR of OEO (DOSTO Powder, Dostofarm GmbH, Westerstede, Germany) (OEO_MR), treatment with 52.5 g/mt SF of a combination of encapsulated and non-encapsulated OEO (DOSTO Ruminant bi-active, Dostofarm GmbH, Westerstede, Germany) (OEO_SF), and treatment with 75 g/mt MR of OEO + 52.5 g/mt SF of OEO (OEO_MR+SF). The groups were compared regarding ADG, feed conversion ratio (FCR) and medication use. The results were analyzed with a linear ANOVA model using R Studio (version 4.3.1) with treatment group as main effect (significance level $P < 0.05$). The ADG of the calves in wk 2–6 did not differ significantly between the groups (CON 966 g/d, OEO_MR 978 g/d, OEO_SF 943 g/d, OEO_MR+SF 980 g/d) ($P = 0.55$). In wk 10–12, there was no significant effect of OEO supplementation on ADG (CON 1,268 g/d, OEO_MR 1,284 g/d, OEO_SF 1,334 g/d, OEO_MR+SF 1,346 g/d). Overall, FCR improved in the OEO-fed groups from wk 0–12, with the OEO_MR+SF group showing a significantly higher FCR compared with the CON group (CON 1.29, OEO_MR 1.24, OEO_SF 1.25, OEO_MR+SF 1.22) ($P < 0.05$). Calves receiving OEO over MR required 17 antibiotic treatments (14 calves) compared with 27 treatments (19 calves) in the group without MR addition. In the critical phase of calf rearing, it might be supportive to start using OEO with the MR from the first days of life and to offer OEO in addition to the SF for optimum FCR.

Key Words: oregano essential oil, calf, feed conversion ratio

2157 Effects of milk replacer fat ratios of coconut oil and palm oil on feeding intake and performance of male dairy calves. J. V. R. Lovatti^{*1}, T. E. da Silva¹, A. J. Keunen³, D. L. Renaud², and J. H. C. Costa¹, ¹University of Vermont, Burlington, VT, ²University of Guelph, Guelph, ON, Canada, ³Maple View Agri, Palmerston, ON, Canada.

The effects of varying coconut-to-palm oil fat ratios in milk replacer (MR) on dairy calves feed intake and performance are unexplored. This study aims on investigate the medium-chain (MCFA) and long-chain fatty acids (LCFA) profile in MR influences on feeding intake and performance of dairy calves. Male Holstein calves (BW = 48.4 ± 4.58 kg; serum total protein [STP] = 5.46 ± 0.59; n = 119) were housed individually for 84-d period, divided in 3 phases: preweaning (1–42 d), weaning (43–63 d), and postweaning (64–91 d). Calves at arrival were randomly assigned to 1 of 3 MR treatments: 1) 20% coconut: 80% palm oil (20%C:80%P; n = 39), low MCFA and high LCFA profile; 2) 35% coconut:65% palm oil (35%C:65%P; n = 38), moderate MCFA and LCFA; and 3) 50% coconut:50% palm oil (50%C:50%P; n = 42), higher MCFA and lower LCFA. The MR was fed twice daily (Mapleview Agri Ltd., ON, Canada; 26% CP; 21% fat; 130 g/L) following the step-up, step-down program: 520 g d 0 to 6, 650 g d 7 to 13, 910 g d 14 to 20, 1,040 g d 21 to 41, 910 g d 42 to 48, 650 g d 49 to 63, daily. Body weight, MR, and calf starter (CS) intake were recorded weekly. The statistical model included fixed effects for treatment, phase, their interaction, and covariates (i.e., initial BW, age, and STP on arrival). Overall, ADG was greater for 35%C:65%P calves during pre-weaning (35%C:65%P = 0.80 ± 0.02, 20%C:80%P = 0.75 ± 0.02, 50%C:50%P = 0.73 ± 0.02 kg/d; $P = 0.03$), and weaning (35%C:65%P = 1.46 ± 0.05, 20%C:80%P = 1.27 ± 0.05, 50%C:50%P = 1.37 ± 0.05 kg/d; $P < 0.01$), with no differences during post-weaning ($P = 0.85$). At d 84, the BW was greater for 35%C:65%P dairy calves (20%C:80%P = 135.90 ± 1.68 kg; 35%C:65%P = 141.60 ± 1.45 kg; 50%C:50%P = 137.18 ± 1.68 kg; $P < 0.01$). Total DMI (MR + CS) was greater ($P < 0.001$) for 35%C:65%P calves due to greater grain intake across all phases ($P < 0.001$), leading to greater metabolizable energy intake for 35%C:65%P calves across all phases (20%C:80%P = 10.11 ± 0.24; 35%C:65%P = 10.81 ± 0.22; 50%C:50%P = 10.43 ± 0.20 Mcal/d; $P < 0.01$). Fat ratios did not affect

feed efficiency (ADG/DMI; $P = 0.55$) or treatment frequency (diarrhea + respiratory diseases; $P > 0.05$). We found that calves fed 35%C:65%P had increased performance, based on a better balance of MCFA and LCFA, by increasing CS intake.

Key Words: nutrient intake, calf, fat source

2158 Calf feeding behaviors after vaccination when fed ad libitum milk from automated milk feeders. B. J. Bone^{*1}, R. E. James², and M. C. Cantor¹, ¹The Pennsylvania State University, State College, PA, ²Virginia Polytechnic Institute and State University, Blacksburg, VA.

Calves deviate from baseline feeding behavior before incurring disease, but it is unknown if a booster vaccination alters their behavior. This case-control study evaluated booster vaccination effects on milk intake, drinking speed, and rewarded visits in Angus × Holstein calves fed milk replacer ad libitum via an automated milk feeder (AMF). We also explored if a calf has relative changes in its feeding behavior relative to the booster (d 0). Calves were sourced from dairies in the United Kingdom and raised by one calf raiser. Feeding behaviors were recorded daily from d -2 to d 4 relative to booster status. Cases were vaccinated with inactive Bovilis Bovivac S (*Salmonella* spp.) within 7 d after arrival and had a booster at 21 d. Control calves received none. We pair-matched booster calves to controls (n = 22 pairs) by sex, the day calves independently drank milk, and weight (SD ± 6 kg). Calves were offered near ad libitum milk intake with a 2-h block between meals; the meal portion maximum was 3 L to prohibit overdrinking. We ran mixed linear regression models and assessed the effects of booster status on calf feeding behavior, relative changes in feeding behavior, and the day × booster status interaction from d -1 to d 4 relative to vaccination with pair as a random effect. Mann-Whitney tests were used to identify a baseline when feeding behavior was not different among booster calves and controls for relative change models (d -2). There was a milk intake × day interaction relative to vaccination status ($P = 0.05$). Vaccinated calves consumed more milk than controls on d 0, 2, 3, and 4 ($P \leq 0.05$). Drinking speed was slightly faster in booster calves (0.53 ± 0.01 L/min) compared with controls (0.50 ± 0.01 L/min; $P = 0.01$). Booster calves had more rewarded visits (4.47 ± 0.16 visits) compared with control calves (3.95 ± 0.16 visits; $P = 0.01$). However, calves had similar relative changes in milk intake, drinking speed, and rewarded visits ($P \geq 0.27$). These results suggest that booster vaccination affected average feeding behavior, but it does not compromise a calf's individual feeding patterns.

Key Words: precision livestock farming, beef on dairy, relative change

2159 Effects of sodium butyrate supplementation on performance and rumen fermentation in dairy calves. L. S. Gheller^{*1}, M. M. Martins², B. L. de Noronha², M. B. Figueiredo¹, A. Saran Netto², and M. S. V. Salles¹, ¹Animal Science Institute (IZ), Ribeirao Preto, SP, Brazil, ²Department of Animal Science, College of Animal Science and Food Engineering, University of Sao Paulo, Pirassununga, SP, Brazil.

The aim of this study was to evaluate the effects of sodium butyrate supplementation on concentrate intake, ADG, and ruminal fermentation in dairy calves. Twenty-four male Holstein calves were blocked according to birth weight and randomly assigned to 2 treatments: control (CON), receiving a basal diet; and sodium butyrate (SB), receiving the same basal diet supplemented with 3 g/kg DM of sodium butyrate in the concentrate. Sodium butyrate supplementation began at 7 d of age and continued until 63 d of age. All calves received 6 L/d of milk replacer

(20.1% CP, 14.1% fat) until d 49, with gradual weaning from d 49 to 63. The concentrate was offered ad libitum and individual intake was obtained daily using an automated feeding system. Calves were weighed weekly. On d 64, 12 calves (6 per treatment) were slaughtered, and ruminal fluid samples were collected. Data were analyzed using the MIXED procedure in SAS 9.4. Block was considered as a random effect, and treatment was included as a fixed effect. Concentrate intake and ADG data were analyzed as repeated measures over time. SB supplementation did not affect concentrate intake (0.303 CON vs. 0.338 SB $\pm$ 0.168 g/d; $P > 0.05$) or ADG (0.496 CON vs. 0.458 SB $\pm$ 0.038 kg/d; $P > 0.05$). Regarding rumen fermentation, SB supplementation increased acetate proportion (51.87 CON vs. 58.40 SB $\pm$ 1.383 mM/100 mM; $P = 0.010$) and the C2:C3 ratio (1.63 CON vs. 2.38 SB $\pm$ 0.168; $P = 0.014$), while reducing propionate proportion (31.43 CON vs. 25.35 SB $\pm$ 1.198 mM/100 mM; $P = 0.007$), without affecting butyrate proportion (9.42 CON, 9.31 SB $\pm$ 0.603 mM/100 mM; $P > 0.05$). Furthermore, there was no effect on total VFA concentration (112.03 CON vs. 113.94 SB $\pm$ 9.83 mM; $P > 0.05$), rumen pH (6.01 CON vs. 6.11 SB $\pm$ 0.148; $P > 0.05$) or N-NH₃ concentration (23.55 CON vs. 19.09 SB $\pm$ 5.61 mg/dL; $P > 0.05$). Therefore, SB supplementation modified rumen fermentation by increasing acetate and the C2:C3 ratio while reducing propionate, but it had no effect on performance.

Key Words: sodium butyrate, rumen fermentation, dairy calf

2160 Effects of age, calf starter intake, and calcified marine algae supplementation on bone development in Holstein calves. S. G. Sayles^{*1}, A. M. E. Hoving^{2,1}, S. M. Kientz¹, M. R. Doschak³, and A. H. Laarman¹, ¹Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, AB, Canada, ²Wageningen University & Research, Wageningen, GD, the Netherlands, ³Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada.

Rib fractures may affect 11% of sick dairy calves and 16% of dairy cows, warranting consideration of factors influencing bone development. Age, feed intake, and nutritional supplements such as calcified marine algae (CMA) may play a role in helping build bone quality. The objective of this study was to determine the effects of age, calf starter intake, and CMA supplementation on frame growth and rib development. Holstein calves (n = 24) were blocked by body weight and randomly assigned to one of 4 treatment groups arranged in a 2 $\times$ 2 factorial design. Calves were fed either calf starter supplemented with limestone (CT; CaCO₃) or 1.25% CMA (CA), and harvested at either 70 d of age (AGE) or 50 kg of cumulative calf starter intake (INT). Animals were fed 900 g/d milk replacer, and underwent a 2-stage stepdown weaning from d 49 to 63. Calf starter was fed ad libitum from d 15. Hip height was measured weekly, and the 11th rib was excised at slaughter for bone structure and mineralization analysis. Ribs were imaged using x-ray micro-CT (mCT; resolution: 18 mm, 90 kV, 278 mA), and reconstructed trabecular bone morphometry was analyzed for bone mineral density (BMD), percent bone volume (%BV), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp). Data were analyzed using the fixed effects of diet and harvest, and random effects of arrival weight and arrival date. Hip height increased weekly from d 21 to 49, and d 56 to 70 ($P < 0.01$), with no effect from diet. Hip height at slaughter was positively correlated with cumulative calf starter intake ($r = 0.25$, $P < 0.01$). Percent bone volume was positively correlated with slaughter age ($r = 0.36$, $P < 0.01$), with no effect from diet or calf starter intake. Trabecular thickness, Tb.Sp, and BMD were not affected by diet, age, or calf starter intake. These data suggest that ad libitum calf starter intake affects frame growth but not bone mineralization, percent bone volume increases with age, and

CMA supplementation may not enhance normal bone development in dairy calves within the first 10 wk of life.

Key Words: calcified marine algae

2161 B-vitamin-related metabolites in plasma from calves fed milk replacers varying in B vitamin concentrations. K. N. Brost^{*1}, M. L. Pister¹, A. Saito², and J. K. Drackley¹, ¹University of Illinois Urbana-Champaign, Urbana, IL, ²The National Federation of Dairy Co-operative Associations (Zen-Raku-Ren), Tokyo, Japan.

Our aim was to compare the concentrations of B-vitamin-related metabolites in plasma from calves fed added B vitamins in milk replacer (MR). Plasma samples were collected from 48 male and female Holstein-Angus cross calves through 43 d of age. Added B vitamins were B₁, B₃, B₆, and B₁₂. Treatments were basal MR with no added B vitamins (CON); MR with B vitamins above basal (BP; 4.27 mg/kg of B₁, 3.86 mg/kg of B₃, 0.54 mg/kg of B₆, and 18 μ g/kg of B₁₂); and MR with B vitamins above the 2021 NASEM recommendations (NP; 28.8 mg/kg of B₁, 18.6 mg/kg of B₃, 9.17 mg/kg of B₆, and 254 μ g/kg of B₁₂). Samples were collected on d 0, 8, 15, 29, and 43 with d 8, 15, 29, and 43 composited. Untargeted metabolomics was performed on d 0 and composite samples by the Carver Metabolomics Core, University of Illinois Urbana-Champaign Roy. J. Carver Biotechnology Center. Metabolite results were analyzed as repeated measures using the MIXED and UNIVARIATE procedures in SAS. Isoleucine and 3-methylhippuric acid were greater ($P < 0.05$), and 2,4-dimethyl nicotinic acid and glycine tended to be greater ($P < 0.1$) for BP compared with CON. The NP calves were greater ($P < 0.05$) for isoleucine, taurocholic acid, methionine, sarcosine, and urate, and tended to be greater ($P < 0.1$) for pyroglu-Trp in comparison to CON. In contrast, His-Ile, tryptophan, pyroglu-Val, and thyroxine were lower ($P < 0.05$), and nicotinamide riboside cation, Asp-Pro, and Gly-Pro tended to be lower ($P < 0.1$) for BP compared with CON. However, no metabolites were lower for NP compared with CON. Greater metabolites could indicate added B vitamins assisted in increasing the metabolism of proteins, fatty acids, and carbohydrates, perhaps improving calf growth and health. Growth and health data from this trial (analyzed separately) showed minimal growth or health improvements for BP and NP. These results indicate that B vitamin inclusion rates for BP and NP could have been greater to match or further exceed the current NASEM requirements. Although this may increase metabolites, it is possible that growth and health may not respond to additional B vitamins.

Key Words: metabolomics, B vitamin, metabolite

2162 Evaluation of crude protein content and rumen unprotected protein sources in diets fed to postweaning heifers. S. Thompson¹, H. Owada², and S. Y. Morrison^{*1}, ¹William H. Miner Agricultural Research Institute, Chazy, NY, ²ZEN-NOH National Federation of Agricultural Cooperative Associations, Tokyo, Japan.

The objective of this study was to evaluate the effect of different dietary protein content and fractions within a compound feed on intake, growth, and feed efficiency in post weaned heifers. Holstein heifers (n = 60; 67 $\pm$ 2 d of age) were blocked by birth date, BW, and age at enrollment in a complete block design. The experimental unit was pen (5 heifers/pen; 4 pens/treatment). Treatments were fed 1 of 3 pelleted compound feeds as grain: 1) 22% CP, low rumen undegradable (30% of CP) and higher rumen degradable protein (70% of CP; 22LRUP) 2) 26% CP, low rumen undegradable sources (26LRUP; 30% of CP) and 26% CP, low rumen undegradable sources (26HRUP; 37% of CP). Treatments and dry hay (16% CP) were offered for ad libitum intake for 11 wk. Intakes (pen)

Table 1 (Abstr. 2162). Average intake growth of heifers in the postweaning period for 11 wk

Item	Treatment (Trt)			SE	P-value		
	22LRUP	26LRUP	26HRUP		Trt	Time	Trt × time
Grain intake, kg/d	4.34	4.44	4.27	0.13	0.66	<0.01	0.12
Hay, kg/d	0.49	0.62	0.62	0.04	0.04	<0.01	<0.01
Grain:hay ratio	9.80	7.70	7.13	0.55	0.02	<0.01	<0.01
BW, kg	151.4	154.4	151.4	1.5	0.40	<0.01	0.67
ADG, kg/d	1.46	1.47	1.47	0.04	0.99	0.03	0.31
Gain:feed, kg/kg	0.33	0.32	0.33	0.01	0.39	<0.01	0.47
BCS	3.15	3.11	3.10	0.02	0.17	<0.01	0.65
Withers height, cm	103.9	104.6	104.4	0.2	0.07	<0.01	0.23
Hip height, cm	108.0	108.4	108.3	0.2	0.33	<0.01	0.16
Heart girth, cm	119.1	120.5	119.1	0.4	0.05	<0.01	0.87
Body length, cm	108.1	109.9	109.2	0.6	0.15	<0.01	0.70
Hip width, cm	33.5	34.1	33.8	0.1	0.02	<0.01	0.35

were recorded daily and BW and stature were recorded weekly for 11 wk. Data were analyzed using the MIXED procedures in SAS. The model included the fixed effect of treatment, time, and interaction. Results are presented in Table 1. The difference in hay intake and grain:hay ratio is thought to be driven by different neutral detergent fiber between CP contents (25% vs. 20% for 22% vs. 26% CP, respectively) in the compound feed, however there could be improved nitrogen efficiency with different protein supply. Sources of RUP, which are often a higher cost, did not seem to affect growth of post weaned heifers when total CP in the compound feed was 26%. With ad libitum intake, there did not appear to be an additional benefit of feeding higher CP content.

Key Words: rumen undegradable, protein, heifer

2163 An analysis of colostrum products on the market in the United States and Japan. H. Owada^{*1}, A. Ideta¹, and S. Y. Morrison², ¹ZEN-NOH National Federation of Agricultural Cooperative Associations, Tokyo, Japan, ²William H. Miner Agricultural Research Institute, Chazy, NY.

There are many colostrum products on the market, and it can be difficult to decide which one to use. To evaluate commercially available colos-

trum products, we purchased 16 different colostrum products sold in the United States (US; n = 7) and Japan (JP; n = 9). The selected colostrum products were marketed and labeled as colostrum replacers (CR; with IgG content ≥60 g/unit) rather than supplements and were divided into 2 types according to their ingredients: (1) colostrum powder (CoP, n = 4); made by drying colostrum, and (2) colostrum mixed (CoM, n = 12), made from multiple ingredients. All products were analyzed (Table 1) for fat, protein, minerals, fatty acids, amino acids, and vitamin A. Effect of CR type and country was evaluated using ANOVA in JMP. Crude protein content ranged from 42% to 60%, fat content from 10% to 20%, and IgG content per bag from 60 to 150 g. The powder content/unit in the US ranged from 225 to 500 g; JP was 225 to 250 g. The content of palmitic (C16) was higher ($P < 0.01$) and oleic (C18:2) was lower in CoP. The Lys:Met was lower in CoP ($P < 0.01$) and had the lowest vitamin A content ($P < 0.05$). CoP had lower vitamin A content because no vitamins and minerals were added, but many CoM samples did add them. Ingredients used in formulation seem to affect the fat and protein contents provided when alternative milk and vegetable ingredients are used compared with drying maternal colostrum. In addition, differences were observed in the fatty acid composition between countries due to the different ingredients used in each country. Each product has its own

Table 1 (Abstr. 2163). Colostrum replacer samples of convenience from the United States (US) and Japan (JP)

Ingredient	CoP			CoM			SE	P-value	
	US (n = 2)	JP (n = 2)	Ave.	US (n = 5)	JP (n = 7)	Ave.		Type	Country
CP (%)	52.45	56.40	54.43	58.30	53.10	55.26	1.69	0.76	0.88
Fat (%)	17.08	14.90	15.99	11.81	11.88	11.85	1.47	0.27	0.77
Ca (%)	0.96	1.46	1.21	0.81	0.64	0.71	0.11	0.06	0.49
P (%)	0.87	0.88	0.87	0.52	0.51	0.51	0.06	<0.01	0.98
C16:0 (%)	42.18	35.32	38.75	27.67	31.53	29.78	1.68	<0.01	0.62
C18:0 (%)	10.51	8.75	9.63	12.38	6.69	9.27	0.91	0.95	0.04
C18:1 (%)	25.59	21.90	23.74	32.77	27.30	29.79	1.70	0.10	0.22
C18:2 (%)	3.30	5.39	4.34	12.39	7.77	9.87	1.02	<0.01	0.39
C18:3 (%)	1.43	0.39	0.91	1.41	0.38	0.85	0.20	0.98	0.01
Lys:Met	3.35	3.27	3.31	4.81	3.93	4.30	0.17	<0.01	0.05
Branched-chain AA (%)	9.84	11.30	10.57	11.37	10.81	11.04	0.33	0.53	0.58
Vitamin A (IU/kg)	22,862	17,225	20,044	57,176	29,749	41,177	5,523	0.04	0.13

characteristics, and it is important to select and use the product that best suits the application.

Key Words: colostrum, replacer, nutrient

2164 Effects of plane of nutrition and inclusion of direct-fed microbials on calf performance and fecal pathogen counts in transportation-stressed calves. T. Forgues^{*1}, B. Juarez¹, D. M. Paulus Compant², and A. J. Carpenter¹, ¹Iowa State University, Ames, IA, ²Papillon Agriculture Company, Easton, MD.

This study examined the effects of differing plane of nutrition (PON) and the inclusion of a direct fed microbial (DFM) containing *Lactobacillus plantarum* and *Bacillus subtilis* species on Holstein calves (n = 76) from birth to 70 d. Treatments were assigned in a 2 × 2 factorial with 2 levels of PON (high [HI] vs. low [LO]), with or without DFM inclusion. Every 5 d, calves (2–7 d of age) were transported for 16 h. Body weight was measured at birth, immediately before and after transportation, and at 24, 48, and 72 h after transportation, as well as 14, 21, 28, 35, 42, 49, 56, 63, and 70 d of age. Fecal samples were collected at the same time points for pathogen analysis, with the exception of birth. Starter and milk replacer (MR) intake was measured daily. HI calves were offered 3.9 kg of a 13.5% solids MR solution twice daily, and LO calves were offered only 2.5 kg of the same solution until weaning at 56 d. Statistical analyses were performed in SAS 9.4 with the main effects of PON, DFM, and their interaction, and PROC MIXED was used with repeated measures for BW, ADG by week, starter intake, MR refusals, and pathogen counts. The model for BW, ADG, and starter intake included Brix score and initial BW. PROC GLM was used to analyze BW postweaning. Preweaning BW was impacted by PON ($P < 0.01$), with higher average BW in the HI group (59.09 ± 0.50 vs. 55.47 ± 0.52 kg; $P \leq 0.01$), but not DFM ($P = 0.55$). Neither PON nor DFM affected ADG ($P \geq 0.17$). PON affected growth in the 2-wk period following weaning ($P = 0.02$), whereas DFM did not ($P = 0.64$). Starter intake was lower for HI (755 ± 27.5 vs. 952 ± 28.0 g/d; $P < 0.01$) but was not affected by DFM ($P = 0.26$). MR refusals were greater in HI calves (14.6 ± 0.01 vs. 5.6 ± 0.1 as % of offered; $P < 0.01$) and unaffected by DFM ($P = 0.64$). There was no evidence of interaction between PON and DFM on any measures. For total fecal pathogen counts, all counts were reduced as calves aged ($P < 0.01$), with no PON or DFM effects on pathogen fecal counts. Overall, PON affected BW and postweaning calf performance.

Key Words: calf, direct-fed microbial, probiotic

2165 Plasma cortisol concentrations and association with stress in weaned calves fed bovine plasma proteins in grower grain. M. L. Pister^{*1}, K. N. Brost¹, J. M. Campbell², and J. K. Drackley¹, ¹University of Illinois at Urbana-Champaign, Urbana, IL, ²APC LLC, Ankeny, IA.

Plasma protein has been shown to help ameliorate stress in preweaned calves, aiding their ability to handle the challenges of weaning and initial social interactions. This study aimed to evaluate stress response of newly weaned calves fed bovine plasma proteins in grower feed. We hypothesized that plasma proteins would reduce plasma cortisol levels in calves during the stress-inducing transition to group housing. In Experiment 1, 21 male Holstein calves were housed outdoors in groups of 3 from 10 to 14 wk of age. Experiment 2 included 40 male and female Holstein-Angus cross calves housed outdoors in groups of 6 or 7 from 9 to 13 wk of age. In both experiments, the calves were weaned on d -1. The weaning process took 1 wk in Exp 1 and 2 wk in Exp 2. Both experiments were randomized complete block designs. Data from

experiments 1 and 2 were pooled. Treatments were a control grower diet with no bovine plasma protein (CON; 20% CP, 2.4% fat, 32% starch) and a grower diet including plasma protein (PP; 20% CP, 2.9% fat, 32% starch). Experiment 1 had 3 CON pens and 4 PP pens. Experiment 2 had 3 CON pens and 3 PP pens. Both experiments provided ad libitum access to water and grower grain, which included 2.5% chopped wheat straw. In Exp. 1, grain was withheld from calves at 1700 h on d 0 to introduce an additional stressor. In Exp. 2, calves completed weaning on d -1 and were moved to group housing on d 0. Data collection was consistent across experiments. Blood samples were collected via jugular venipuncture on d 0 (Exp. 1) or -1 (Exp. 2), d 3, and d 7 (Exp. 1) or d 6 (Exp. 2); plasma was stored at -20°C for later analysis. Plasma was analyzed for cortisol concentrations using an ELISA assay. Data were analyzed using the MIXED and UNIVARIATE procedures in SAS. Feed intake, BW, and ADG did not differ among treatments. No differences in cortisol concentrations (1.05 ng/mL, SEM = 0.03 for CON and 1.07 ng/mL, SEM = 0.03 for PP) were observed between CON and PP calves. Potential stressors associated with weaning did not cause an elevation in plasma cortisol, and dietary plasma protein did not affect growth or cortisol concentrations.

Key Words: plasma protein, cortisol, stress

2166 Evaluation of astaxanthin from *Haematococcus pluvialis* and olive biochar in preweaning Holstein calves: Effects on gut health, metabolic profile, and litter emissions. E. Scaglia^{*1}, V. Sergi¹, L. Giagnoni¹, D. Casini², D. Chiaramonti^{2,3}, G. Gilioli¹, and V. Caprarulo¹, ¹Department of Civil Engineering, Architecture, Environment, Land Planning and Mathematics (DICATAM), University of Brescia, Brescia, Italy, ²RE-CORD (Renewable Energy Consortium for Research and Demonstration), Scarperia e San Piero, Italy, ³Department of Energy (DENEG), Politecnico di Torino, Torino, Italy.

The aim was to investigate the effects of astaxanthin and biochar on diarrhea incidence, metabolic profile, and litter emissions in preweaning female Holstein calves. Calves (37.03 ± 3.89 kg) were assigned to a control (CTRL, n = 12), astaxanthin (AXA, n = 12) or biochar (CHAR, n = 12) group. CTRL was fed with basal diet (milk + ad libitum feed), AXA was fed with basal diet plus 40 mg/d from d0 to 28, and 80 mg/d from d29 to 56 of astaxanthin, and CHAR was fed with basal diet plus 4 g/d from d0 to 28, and 10 g/d from d29 to 56 of biochar. Milk intake (MI), feed intake (FI), and fecal consistency score (FS) were recorded daily. Body weight was recorded weekly, individual fecal samples were collected and analyzed for total, *Lactobacillus* spp., and coliform bacteria on d 0, 28, and 56. Blood samples were collected and analyzed for metabolic profile on d 0 and 56. On d 56, the litter (feces, urine, straw) of CTRL and CHAR were collected from individual boxes and pooled into dynamic chambers (CTRL = 6, CHAR = 6) to simulate storage. Emissions of CO₂, CH₄, N₂O, NH₃ (g/m² per hour) were monitored for 28 d by Gasmeter-GT5000. Data were analyzed by PROC GLIMMIX and PROC FREQ (SAS 9.4). Feed intake was significantly higher in CHAR ($P < 0.001$); no differences in MI and BW were observed. Overall, there were lower diarrhea frequencies in AXA and CHAR than CTRL (-13.5% and -7.07%, respectively; $P < 0.01$). Coliform count in feces was lower in AXA at d56 ($P < 0.05$), no differences were detected for total bacteria and *Lactobacillus* spp. Blood α -2 globulin was higher in CHAR than CTRL ($P < 0.01$). At d 56, CO₂ (-29%, $P < 0.01$) and CH₄ (-15%, $P < 0.05$) were significantly lower in CHAR litter, while overall emissions of CO₂ ($P < 0.01$) and CH₄ ($P < 0.05$) were higher in CHAR. Overall, there was a reduction by 20% for N₂O ($P < 0.05$) and by 46% for NH₃ ($P < 0.001$) in CHAR. In conclusion, the supplementation of AXA and CHAR showed beneficial effects on diarrhea reduction and

coliform presence, and CHAR modulated GHG and ammonia emissions, comparable with organic matter fermentation and litter stabilization.

Key Words: preweaning calf, astaxanthin, biochar

2167 Feeding behavior of dairy calves fed forage sources in total mixed ration. A. F. Toledo, C. R. Tomalusi, N. I. Oliveira, J. G. Dantas, and C. M. M. Bittar*, *Department of Animal Sciences, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, Sao Paulo, Brazil.*

Forage provision encourages early feed intake and supports the transition during the weaning. However, the influence of forage sources in TMR on feeding behavior must be better understood. This study aimed to understand the feeding behavior of calves fed different forage sources in TMR. Calves ($n = 48$) were individually housed, blocked, and randomly assigned to different diets: a no-forage starter (CON); or total mixed rations containing 7.5% on DM basis of Tifton hay, either medium quality (HMQ) or low quality (HLQ); or 10% on DM basis of corn silage (CS), 12 calves per treatment. Calves were fed 6 L/d of whole milk, a commercial starter with no forage, and water until 28 d of age when the solid diet was changed to the respective treatments. Calves were gradually weaned on 56 d and followed for 14 d after weaning. The intake of TMR offered and theorts following 10 h of feed access were measured on d 49 (preweaning) and 70 (postweaning), and the behavior was recorded. Orthogonal contrasts were used to determine the effects between control $\times$ all forage containing TMR, corn silage $\times$ hay, medium quality hay $\times$ low-quality hay. There was no diet effect on TMR intake ($P > 0.05$). However, the forage provision increased NDF intake ($P < 0.05$), and the inclusion of hay, HMQ and HLQ, tended to increase NDF intake compared with CS diet ($P < 0.08$) at d 49 and 70. The same effect was observed for physically effective NDF >4 mm intake. There was no diet effect on TMR intake variation at wk 7, but at wk 10, calves fed a CON diet tended to present greater variation than calves fed forage ($P < 0.07$). On d 49, forage provision increased the time spent chewing, the number of meals, and the average time spent on each meal ($P < 0.05$). The same effect was observed at d 70; however, the CS diet increased the average time spent in each rumination cycle ($P < 0.05$). These results suggest that the fiber source in the TMR influences the intake capacity and the ingestive behavior of dairy calves before and after weaning. In addition, the solid feed intake variation during preweaning suggests that early exposure to forage, irrespective of the feeding strategy, is crucial to support the transition to the next phase.

Key Words: short-term effect, hay, corn silage

2168 Combinations of different liquid and solid diets and the performance and health of dairy calves. C. M. M. Bittar*, C. R. Tomalusi¹, J. M. Feliciano¹, E. D. Marino¹, J. R. A. Castro¹, I. C. R. Oliveira¹, N. I. Carvalho¹, G. B. Baggi¹, M. H. Mortazavi², C. Hilgert³, and E. W. Carneiro³, *¹Department of Animal Sciences, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, Sao Paulo, Brazil, ²Department of Animal Science, University of Tehran, Karaj, Alborz, Iran, ³Agrifirm Brasil, Curitiba, Paraná, Brasil.*

Liquid and solid diets with different qualities and physical form can result in similar performance depending on intake adjustments by dairy calves. This study aimed to evaluate the association of liquid diet (LD) alternatives with different concentrates (SD) and their effects on preweaning performance and health. Sixty Holstein calves were used in a 2×2 factorial design, with 2 sources of LD (milk replacer [22% CP and 17% fat] diluted to 15% solids [MR] or powdered whole milk

[24.5% CP and 26.5% fat] diluted to 12.5% solids and added with 25 g/L of milk replacer to achieve 15% solids [WM+]); and 2 types of SD (micropellet [Micro] or textured [Text]), both with 22% CP and 10% ADF. Concentrates were based on ground corn and soybean meal, but whereas Micro presented soybean hulls, Multi presented extruded and steam-flaked corn and shelled oats. Metabolizable energy of the MR was 4.3 Mcal/kg and 4.69 Mcal/kg for the WM+. and ingredients of SD were not equal. Calves were individually housed and fed 8 L/d until wk 3 and then 6 L/d of the respective LD until 56 d of age when gradual weaning began by reducing volume by 1 L/d. Calves had free access to water and the respective SD. Hay was fed ad libitum from the seventh week. Data were analyzed using the MIXED procedure of SAS, with treatments as fixed effect, and the means were compared using Tukey test. Orthogonal contrasts were built to test the effect of LD and SD. Liquid diet and hay DMI were not affected by treatments ($P > 0.05$). However, concentrate and total diet DMI were higher for calves fed MR and Text compared with WM+ and Micro ($P < 0.05$). Orthogonal contrast for SD shows higher concentrate and total diet DMI for calves fed Text ($P < 0.01$). Final weight was not affected by treatments, but greater ADG was observed for calves fed WM+ and Text compared with those fed MR, regardless of the concentrate, with no differences with WM+ and Micro ($P < 0.05$). Orthogonal contrast of LD showed lower ADG ($P < 0.01$), and lower hip width ($P < 0.05$) and a trend for lower withers height and heart girth gains ($P < 0.07$) for calves fed MR. Feed efficiency was not affected by treatments ($P > 0.05$). Fecal score, days with fecal score >2 , and days to the first episode of diarrhea suggest greater challenge for calves fed MR ($P < 0.003$), regardless of the starter feed. Even with adjustments on intake, the supply of WM+ combined with the Text concentrate resulted in greater performance.

Key Words: milk replacer, high solids, micropellet

2169 Combinations of different liquid and solid diets: Rumen fermentation and gastrointestinal tract development. C. M. M. Bittar*, E. D. Marino¹, J. M. Feliciano¹, C. R. Tomalusi¹, J. R. A. Castro¹, F. V. L. Barbosa¹, B. R. O. Silva¹, M. N. Favarin¹, M. H. Mortazavi², C. Hilgert³, and E. W. Carneiro³, *¹Department of Animal Sciences, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, Sao Paulo, Brazil, ²Department of Animal Science, University of Tehran, Karaj, Alborz, Iran, ³Agrifirm Brasil, Curitiba, Paraná, Brasil.*

Solid diet (SD) intake is crucial for gastrointestinal tract (GIT) development. However, intake is a function of liquid diet (LD) feeding management and composition and intrinsic factors of SD such as nutrient composition and physical form. This study aimed to evaluate the association of LD alternatives with different SD and their effects on rumen fermentation profile and GIT development. Sixty Holstein calves were used in a 2×2 factorial design, with 2 sources of LD (milk replacer [22% CP and 17% fat] diluted to 15% solids [MR] or powdered whole milk [24.5% CP and 26.5% fat] diluted to 12.5% solids and added with 25 g/L of milk replacer to achieve 15% solids [WM+]); and 2 types of SD (micropellet [Micro] or textured [Text]), both with 22% CP and 10% ADF. Calves were individually housed and fed 8 L/d until wk 3 and then 6 L/d of the respective LD until 56 d of age, when gradual weaning began by reducing volume by 1 L/d, and had free access to water and the respective concentrate. Hay was fed ad libitum from the seventh week. Eight calves/treatment were used for rumen content sampling with an oro-esophageal tube, 2 h after feeding, at wk 7, 8 and, 9 of age; and 4 calves/treatment were slaughtered at wk 9. Data were analyzed using the MIXED procedure of SAS, with treatments as fixed effect, and the means were compared using Tukey test. Orthogonal contrasts were

built to test the effect of LD and SD. A higher rumen pH was observed for calves fed WM+ and Text compared with calves fed MR, regardless of the SD, with no differences with WM+ and Micro ($P < 0.001$). Calves fed WM+ and Text presented the highest pH at wk 8, with no differences among treatments in the other evaluated weeks. The molar concentration of VFA was affected by age with the highest concentrations at wk 9. The molar proportion of propionic acid was higher for calves fed MR and Micro compared with calves fed WM+ and Text, with no differences with other treatments. C2:C3 and C2:C3+C4 ratios were not affected ($P > 0.05$). GIT compartments weight or in % of BW, GIT fill, and average number of papillae per cm^2 were not affected by treatments. Differences in rumen fermentation profile were not enough to affect GIT development.

Key Words: papillae, milk replacer, micropellet

2170 Derivation of the maintenance energy requirements in growing Jersey heifers. A. L. Carroll^{*1}, M. J. VandeHaar², and P. J. Kononoff¹, ¹University of Nebraska-Lincoln, Lincoln, NE, ²Michigan State University, East Lansing, MI.

Maintenance energy requirements for growing dairy heifers have not been experimentally studied in great depth since the mid-1980s, and to our knowledge, no direct measurements of maintenance have ever been conducted on growing Jersey heifers. The maintenance energy requirement of growing animals is first fulfilled before energy use for gain. As a result, current estimates of maintenance are needed to effectively model gain. Therefore, the objectives of this study were to first use indirect calorimetry to estimate maintenance energy requirements and fasting heat production (FHP) of growing Jersey heifers, and second to compare NEM via indirect methods of regression from the data associated with ME for maintenance (ME_{maint}) versus that estimated using direct measures fasting heat production. Eight nonpregnant growing Jersey heifers (9.53 ± 0.114 mo, 239 ± 14.5 kg) were enrolled in the study and fed at maintenance estimated from NASEM (NEM [Mcal/d] = $0.10 \times \text{BW}^{0.75}$) for 24 d; after which energetic measures were made for 4 d including indirect calorimetry, intake, and total feces and urine excretion. Heifers were then fasted for 96 h with measures once again taken over the final 24 h. Heat production for growing heifers at NASEM model-calculated maintenance intake averaged 9.55 ± 0.608 Mcal/d, and fasting heat production was observed to be 6.56 ± 0.444 Mcal/d. This resulted in the following representation of maintenance energy requirements; ME_{maint} (Mcal/BW^{0.75} $\pm$ SD) = 0.157 ± 0.0056 and NEM (Mcal/BW^{0.75} $\pm$ SD) = $0.108 \pm 0.0051 \times \text{BW}^{0.75}$. The efficiency of energy conversion of energy for maintenance (k_M) averaged 0.684 ± 0.0410 . Thus, these estimates of maintenance support those of NASEM (2021), although they are slightly higher.

Key Words: growing heifer, energy utilization, fasting heat production

2171 Differences in colostrum and milk replacer supply influence gastrointestinal permeability in young Holstein calves. J. Echeverry-Munera^{*1,2}, M. A. Steele², L. N. Leal¹, and J. Martin-Tereso¹, ¹Trouw Nutrition R&D, Amersfoort, the Netherlands, ²Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.

This study examined early-life nutritional interventions in their effects on gastrointestinal permeability over time in male Holstein calves ($n = 180$; 43.1 ± 3.56 kg BW). In the first 12 h after birth, differences in passive immunity transfer were induced by feeding either 2 L (Failure [F]; 100 g of IgG) or 6 L (Successful [S]; 300 g of IgG) of colostrum replacer (CR). Within 3 d, calves were transported to the research facility,

where serum IgG was measured. Calves were blocked by CR protocol, arrival date and age, and within block randomly assigned to 2 milk replacer (MR) volume treatments: moderate (M; 0.250 Mcal of ME/kg of arrival BW^{0.75}) or low (L; 0.135 Mcal of ME/kg of arrival BW^{0.75}). This resulted in a 2×2 factorial design with 4 treatments: FM, FL, SM, SL ($n = 45/\text{trt}$). Calves were individually housed, fed twice daily until d 42, gradually weaned over 2 wk and fully weaned by d 57. Calves had ad libitum access to straw and water, and from d 22 on, a pelleted calf starter was offered. Gastrointestinal permeability was assessed at wk 3, 6, and 12 by orally dosing 15 calves per treatment with Cr-EDTA, followed by blood sampling at 0, 90, 120, 180, 300, 360 and 420 min relative to marker administration to determine serum Cr appearance. Data were analyzed using PROC GLIMMIX in SAS, including the fixed effects of CR, MR, age, and their interaction, with block as a random effect. At wk 3, a CR $\times$ MR interaction effect was observed on Cr area under the curve (AUC; $P = 0.05$), with SM calves having the lowest value, while SL calves had the greatest Cr AUC. Maximum serum Cr concentration (C_{max}) also showed an CR $\times$ MR interaction effect ($P = 0.04$), with SL calves having the highest peak of Cr concentration. Time to peak serum Cr concentration (T_{max}) showed a significant CR treatment effect at wk 12 ($P = 0.04$), with S calves reaching their C_{max} sooner than F calves. Overall, AUC, C_{max} and T_{max} were affected by age ($P < 0.01$), with the highest values being observed at 3 wk of age. These results suggest that colostrum and MR feeding supply can influence gut permeability in young calves, with potential implications for nutrient absorption and gut barrier function. It was also observed that permeability follows a developmental decline, with treatment effects becoming less pronounced with age.

Key Words: gastrointestinal permeability, early-life nutrition

2172 Growth performance and health of calves fed milk replacers formulated with tailor-made soy protein concentrate. L. Zou^{*1}, A. Keunen², and D. Wood³, ¹Bunge North America, St. Louis, MO, ²Mapleview Agri Ltd., Palmerston, ON, Canada, ³Animix LLC, Juneau, WI.

The objective of this study was to evaluate PurePro soy protein concentrate (SPC) with tailor-made physical and nutritional properties for CMR on calf growth and health at a commercial calf raising facility. A total of 192 male Holstein ($n = 145$) and crossbred ($n = 47$) calves at 5 to 10 d of age from local farms ($n = 75$) and auction ($n = 117$) were randomly assigned to 3 treatments ($n = 64$): 1) all milk CMR (ALLMK), 2) CMR with 5% SPC (SPC5); and 3) CMR with 5% SPC and 5% bovine plasma (SPC/BP). All diets were standardized to 26% protein and 20% fat with 2.4% lysine, 0.8% methionine and 1.6% threonine. Calves were housed individually and fed 6 L of CMR twice daily from d 0 to 14 followed by 8 L of CMR until d 42 with a gradual weaning occurring from d 42 to 63. All statistical analyses were conducted on Stata 18, with a combination of repeated measures linear regression, linear regression, negative binomial models, logistic regression, and Cox proportional hazard models were used to evaluate treatment effects on growth, feed intake, efficiency, health outcomes, and mortality over the experimental period. Overall, groups experienced a higher mortality (12%; $P = 0.58$) due to a *Histophilus somni* outbreak during the trial. Compared with ALLMK (29.7%) and SPC/BP (28.1%) groups, SPC5 group had a numerically higher number of calves (43.8%; $P = 0.12$) with failed transfer of passive immunity (serum total protein < 5.2 g/dL) at arrival which was found to significantly ($P < 0.0001$) affect mortality, respiratory disease, grain and water intakes, and growth (BW, ADG). There were no significant differences in respiratory disease ($P = 0.22$) among treatments, although time and treatment interaction ($P = 0.0007$)

was found. No significant differences were observed in diarrhea fecal scores ($P = 0.46$), CMR intake ($P = 0.52$), water intake ($P = 0.78$), and grain intake ($P = 0.42$) over the entire period. No differences were found in ADG over the 84-d experimental period, except an interaction ($P = 0.01$) was found between the calf breed (Holstein) and treatment groups at the post-weaning. At d 70, calves fed SPC5 tended ($P = 0.10$) to have gained 2.72 kg less body weight compared with ALLMK, whereas at d 84, calves fed SPC5 had gained 3.36 kg less body weight ($P = 0.04$). No differences in feed efficiency were found between SPC5 and ALLMK, whereas calves fed SPC/BP required 0.09 kg more of feed per kg of body weight gain over the entire period ($P = 0.05$). In conclusion, utilizing 5% PurePro SPC in milk replacer formulation had minimal effects on the health and performance on calves at a commercial calf rearing facility; however, the effect on future performance is needed.

Key Words: soy protein concentrate, milk replacer, calf

2173 Effects of adding of ethyl esters of polyunsaturated fatty acids of linseed oil or linseed oil to milk replacer on nutrient digestibility in dairy calves. M. K. Baba^{*1,2}, J. Flaga¹, and Z. M. Kowalski¹, ¹University of Agriculture in Krakow, Krakow, Poland, ²Nasarawa State University, Keffi, Nigeria.

The aim of this study was to determine the effects of adding of ethyl esters (EE) of polyunsaturated fatty acids (PUFA) of linseed oil or linseed oil (LO) to milk replacer on nutrient intake and digestibility coefficients in dairy calves. A total of 36 Holstein Friesian dairy calves were divided into 3 groups (12 animals each): control (no source of PUFA added to MR), linseed oil (LOG; 30 mL/d LO added to MR), and ethyl esters group (EEG; 30 mL/d EE added to MR) groups. The animals were fed MR with or without the added source of PUFA and titanium dioxide as the marker. The experiment had 2 phases (first month and second month of animals' life), 6 d adaptation period and 4 d of fecal collection in each. Total dry matter intake tended (g/d ; $P = 0.10$) to differ between the groups (mean $\pm$ SEM; control: $1,121.91 \pm 40.53$; LOG: $1,162.54 \pm 43.66$; EEG: $1,265.97 \pm 45.90$). There was no difference in average daily gain (g/d ; $P = 0.19$; control: 629.65 ± 34.12 ; LOG: 668.64 ± 36.76 ; EEG: 734.30 ± 38.64) and feed efficiency ($P = 0.79$; control: 0.56 ± 0.02 ; LOG: 0.58 ± 0.02 ; EEG: 0.59 ± 0.03) between the groups. Organic matter intake (g/d) was higher ($P = 0.02$) in EEG (662.08 ± 18.79) than in the control (570.11 ± 16.59) and tended to be higher in animals in LOG (610.65 ± 17.87). Crude fat intakes (g/d) of LOG (184.86 ± 2.79) and EEG (190.31 ± 2.93) supplemented animals were not different but were higher ($P < 0.0001$) than the control (150.70 ± 2.59) group animals. Dry matter digestibility (%) tended ($P = 0.09$) to be different between the groups (control: 87.70 ± 1.29 ; LOG: 85.14 ± 1.39 ; EEG: 90.12 ± 1.46). In conclusion, adding ethyl esters of polyunsaturated fatty acids of linseed oil showed potential for

improved intakes and nutrient digestibility in the diets of dairy calves within their first 2 mo of life.

Key Words: calf, dairy, fatty acids, digestibility, ruminant

2174 Monitoring the effects of oxidative stress on the growth of Holstein bull calves using Diquat. D. P. Casper^{*1,2} and T. Liu³, ¹Casper's Calf Ranch LLC, Freeport, IL, ²North Carolina A&T State University, Greensboro, NC, ³Gansu Agricultural University, Lanzhou, China.

Oxidative stress is defined as excess (imbalance) free radical concentrations leading to intestinal dysfunction. A total of twelve 70-d-old Holstein bull calves with similar BW and body condition were randomly assigned to one of 4 treatments. The one-time treatments were as follows: control: calves were injected with 0 mg/kg BW Diquat in 0.9% sterilized saline; treatments 6, 8, and 10 mg/kg BW Diquat, respectively. The experimental period lasted for 24 d. Measurements of BW, ADG, fecal scores, frame gains, fecal pathogen count, and blood samples for monitoring oxidative stress were collected on d 0, 6, 12, 18, and 24. Data were analyzed using a randomized complete block design, with days considered a repeated measurement. In addition, orthogonal polynomial contrasts were used to assess the linear, quadratic, and cubic treatment responses. Growth performance (BW) and ADG demonstrated a cubic response ($P < 0.01$) initially decreasing and then increasing with higher Diquat dosages. Fecal scores and fecal ratios (number of diarrheic calves divided by total calf numbers) exhibited a quadratic ($P < 0.02$) response rising at a diminishing rate as Diquat injection dosages increased. Frame gains for body slope (anterior scapula to posterior ipsilateral sciatic tubercle), body length, hip height, and abdominal girth displayed a linear ($P < 0.01$) decrease with increasing Diquat injection dosages. Serum aspartate aminotransferase, glutathione, total antioxidant capacity, catalase, malondialdehyde, cortisol, and noradrenaline concentrations revealed a linear ($P < 0.01$) increase in response to higher Diquat injection dosages, whereas alanine transaminase, superoxide dismutase, and glutathione peroxidase demonstrated a quadratic ($P < 0.02$) response, increasing at a diminishing rate. Fecal *Escherichia coli* concentrations demonstrated a cubic ($P < 0.01$) response, and *Staphylococcus aureus* and *Salmonella-Shigella* demonstrated linear ($P < 0.01$) increases with increasing Diquat dosages. Diquat injection induced oxidative stress, leading to reduced growth performance, along with increased serum oxidative stress indices, fecal scores, and fecal pathogens, a response that may persist for up to 24 d. An optimal dosage of 8 mg/kg BW is proposed as a benchmark for elucidating oxidative stress to evaluate future technologies aimed at reducing, eliminating, or preventing oxidative stress.

Key Words: Diquat, oxidative stress, weaned calf

Ruminant Nutrition 1: Carbohydrates—Posters

2175 Effects of glycan supplementation on intake, performance, rumen fermentation, and blood parameters in early-lactation dairy cows. O. Javed*, K. Arriola, S. Saikumar, N. Alfaro-Diaz, M. U. Siregar, F. T. Saputra, C. D. Nelson, F. Batistel, and D. Vyas, *University of Florida, Gainesville, FL*.

We aimed to evaluate the effects of dietary supplementation of glycan on DMI, production performance, blood metabolites, and rumen fermentation profile for 14 weeks (2 weeks covariate and 12 weeks treatment period) in early-lactating dairy cows. Eighty-four multiparous lactating Holstein dairy cows were enrolled around d 21 and blocked by energy corrected milk after 2 weeks of covariate period. Following treatments were randomly allotted to each block ($n = 21/\text{treatment}$): 1) control—cows fed corn silage based TMR without glycan supplementation; 2) Low—26.7 ppm glycan mixed with the TMR; 3) Med—124.4 ppm glycan mixed with the TMR; and 4) High—244.4 ppm glycan mixed with the TMR. Diets were formulated using CNCPS v.6.5.5. for cows producing 38.5 kg/d with 3.70% milk fat and 3.10% true milk protein and expected DMI of 21 kg/d. Data were analyzed using GLIMMIX procedure of SAS using treatment, week and their interaction along with block and covariate parameters as fixed factors. Cows nested in treatment was used as random factor and week was used as repeated measure. Control treatment was compared with Low, Med, and High using contrast statement and significance was declared at $P < 0.05$ and as trends at $0.05 \leq P < 0.10$. No treatment effect was observed on DMI, milk yield (MY), 3.5% fat-corrected milk (FCM), and energy-corrected milk ($P > 0.10$) and milk yield ($P > 0.10$). However, feed efficiency (MY/DMI) was numerically greater with High ($P = 0.11$), whereas feed efficiency corrected for FCM (FCM/DMI; $P = 0.15$) and ECM (ECM/DMI; $P = 0.16$) was numerically greater with Med when compared with CON. No treatment effect was observed on the concentration and yield of milk protein and milk fat. In conclusion, medium and high levels of Glycan supplementation may have potential for improving feed efficiency in dairy cows; however, further research is needed to determine the optimal dosage for maximizing the efficacy and consistent performance benefits with glycan supplementation.

Key Words: glycan, dairy cow, performance

2176 Evaluating the effects of an oral bolus containing live yeast, Ca, Mg, and P on the severity of a high-starch-diet challenge. A. Fraz*, F. T. Saputra¹, Z. Sarwar¹, M. C. Perdomo¹, N. C. Upah², and C. D. Nelson¹, ¹University of Florida, Gainesville, FL, ²TechMix LLC, Stewart, MN.

Objectives were to assess the effects of an oral bolus containing live yeast, Mg, Ca, and K (YMCP Vitall) on feed restriction model design to introduce subacute ruminal acidosis induced through an abrupt change to a high starch diet. The hypothesis was that bolus treatment would reduce the severity of subacute ruminal acidosis by decreasing inflammation and improving feed intake. Pregnant nulliparous cows ($n = 24$) were fed a low starch diet (~15% starch, DM; forage-to-concentrate ratio = 65:35) for 7 d, followed by reducing feed intake by 10% each day for 3 d, and then offered a high starch diet (~32% starch, DM; forage to concentrate ratio = 40: 60) ad libitum for 7 d. Cows were blocked based on DMI and body weight and randomly assigned to receive either no bolus (CON) or YMCP Vitall bolus (BOL) treatment at 0 and 12 h relative to offering the high starch diet. Data were analyzed using linear mixed-effects models

through the MIXED procedure of SAS, incorporating fixed effects of treatment, time and their interaction. Dry matter intake (CON = 11.2 vs. BOL = 12.4 ± 0.48 , kg/d) did not differ between treatments. The BOL treatment reduced ($P = 0.04$) serum Hp concentrations compared with CON (CON = 386 vs. BOL = 110 ± 88 , $\mu\text{g/mL}$). Additionally, a treatment by time interaction ($P = 0.05$) was found, as BOL reduced serum Hp concentrations on hours 48, 72, and 96, relative to high starch diet. An interaction between treatment and time ($P = 0.04$) also affected glucose concentrations because plasma glucose was greater at 12 h for cows receiving the BOL treatment compared with CON (CON = 6.36 vs. BOL = 7.02 ± 0.28 , mM); whereas BOL decreased plasma glucose compared with CON at 72 and 96 h after introduction of the high starch diet (72 h: CON = 6.78 vs. BOL = 5.91 ± 0.28 , mM; 96 h: CON = 7.07 vs. BOL = 6.12 ± 0.28 , mM). In conclusion, BOL markedly reduced serum Hp concentrations and altered blood glucose dynamics in cows challenged with decreasing feed intake followed by an abrupt conversion to a high starch ad libitum diet.

Key Words: mineral yeast inflammation

2177 Effects of *brachytic2* mutation in corn silage on performance and total-tract digestibility by lactating Holstein dairy cows. E. Sarmikasoglou*, D. Hammer², T. Dietz², and M. J. Vande-Haar¹, ¹Michigan State University, East Lansing, MI, ²Bayer Crop Science, Chesterfield, MO.

Our objective was to determine if diets containing 3 novel corn silage (CS) hybrids with short stature genetics (SH-1,2,3) would increase milk production of Holstein cows compared with diets containing tall (TALL) or *bm3* (BMR) CS hybrids. These short stature corn hybrids carry the *brachytic2* (*br2*) mutation, which results in reduced plant height and improved standability. Twenty primiparous and 20 multiparous Holstein cows (150 ± 42 DIM, 35 ± 6 kg milk/d, 674 ± 103 kg BW) were fed a common diet for 14d and then enrolled in an incomplete Latin square design with three 21-d periods. Experimental diets contained CS from TALL, BMR, SH1, SH2, or SH3, along with alfalfa silage, soybean meal, soybean hulls, ground corn, and a premix of protein, minerals and vitamins. All diets were formulated for 18% CP and 24% for NDF and fed as TMR. Data were analyzed using PROC GLIMMIX of SAS. Pre-planned contrasts were BMR vs. non-BMR (BMR vs. TALL, SH-1,2,3); TALL vs. Short (TALL vs. SH-1,2,3); and BMR vs. Short (BMR vs. SH-1,2,3). Cows fed BMR CS diet had lower DMI (24.5 vs. 26.4 kg; $P < 0.01$), milk fat percent (4.4% vs. 4.3%; $P < 0.01$), and yields of ECM (36.5 vs. 37.5 kg/d; $P = 0.02$) and protein (1.1 vs. 1.14 kg/d; $P < 0.01$), and greater feed efficiency (1.51 vs. 1.43; $P < 0.01$) than did cows fed non-BMR hybrids. Compared with cows fed the diet with TALL, cows fed diets with Short CS produced more milk (32.5 vs. 33.2 kg/d; $P = 0.02$), protein (1.11 vs. 1.15 kg/d; $P < 0.01$), and lactose (1.56 vs. 1.61 kg/d; $P < 0.01$), but had lower fat percent (4.43% vs. 4.32%; $P < 0.01$). Compared with cows fed BMR CS, cows fed diets with Short CS had greater DMI (24.5 vs. 26.5 kg; $P < 0.01$) and yields of milk (32 vs. 33 kg/d; $P < 0.01$), ECM (36.5 vs. 37.6 kg/d; $P = 0.01$), and protein (1.1 vs. 1.15 kg/d; $P < 0.01$), but lower fat percent (4.45% vs. 4.32%; $P < 0.01$) and feed efficiency (1.51 vs. 1.43; $P < 0.01$). Apparent total-tract digestibility of starch (98.1% vs. 99.2%; $P < 0.01$) and CP (67.2% vs. 71.1%; $P < 0.01$) was lower in cows fed BMR CS than those fed non-BMR CS. In conclusion, feeding diets with *br2* CS to cows in mid-/

late-lactation enhances feed intake and milk production compared with diets with BMR or TALL CS.

Key Words: fiber, *br2*

2178 Effects of corn silage and grain expressing α -amylase on productive performance and digestibility in dairy cows. F. P. Rennó^{*1}, M. S. C. Kohlmann¹, B. C. Costa¹, R. F. Galdino¹, N. T. S. Grigoletto¹, A. J. C. Oliveira¹, G. G. da Silva¹, and C. S. Takiya^{2,1}, ¹University of São Paulo, Pirassununga, São Paulo, Brazil, ²Universidade Tecnológica Federal do Paraná, Pato Branco, Paraná, Brazil.

Corn with increased activity of α -amylase (Enogen corn; Syngenta Seeds LLC) has the potential to increase starch availability compared with conventional corn. An experiment was conducted to evaluate the effects of corn grain (CG) and corn silage (CS) with enhanced endogenous α -amylase activity on milk yield efficiency and apparent total-tract nutrient digestibility. Twenty-four Holstein cows (35.4 ± 4.25 kg/d milk yield, 130 ± 56 DIM) were blocked by parity and assigned to a 4×4 Latin square (LS) experiment with 21-d periods, and a 2×2 factorial treatment arrangement. Cows were randomly assigned to one of the following treatments: (1) a diet containing isoline CS and isoline CG (CON); (2) a diet with isoline CS and Enogen CG (ECG); (3) a diet with Enogen CS and isoline CG (ECS); and (4) a diet with Enogen CS and Enogen CG (ECSCG). Isoline refers to the isogenic counterpart of Enogen corn grain or silage. Enogen and isoline corn were planted, harvested, and processed for silage and grain equally. Samples of TMR, orts, and feces were analyzed for chemical composition. Fecal samples were collected for 3 consecutive days of each period at 9 h-intervals. Fecal excretion was estimated based on indigestible NDF. Cows were milked twice daily and samples were collected during 6 consecutive milkings. Data were analyzed using the MIXED procedure of SAS modeling the fixed effects of treatment, period, LS, and their interaction. Animal within LS was considered a random effect. DMI and digestibility of DM, OM, CP, and starch were similar among treatment groups. The digestibility of NDF was higher ($P = 0.05$) in ECS treatments. 3.5% FCM yield ($P = 0.03$; 34.3, 35.0, 35.3, and 36.4 kg/d for CON, ECG, ECS, ECSCG, respectively) and fat yield ($P = 0.03$; 1.21, 1.28, 1.29, and 1.35 kg/d for CON, ECG, ECS, ECSCG, respectively) were increased in ECS diets. ECS diets tended to increase milk yield and ECG diets tended to increase 3.5% FCM yield. Feed efficiency (FCM/DMI) was increased ($P = 0.04$) in ECS diets. No interaction was observed between ECS and ECG diets for milk yield and composition. Feeding ECS improved milk and fat yield and efficiency in cows.

Key Words: alpha-amylase, Enogen corn

2179 Physically adjusted neutral detergent fiber system: Effects of dextrose and nonforage fiber on performance, feeding behavior, digestibility, and rumen fermentation in dairy cows. J. B. Urbina¹, M. R. A. Redoy^{*2}, and M. E. Uddin², ¹Dairy and Food Science Department, South Dakota State University, Brookings, SD, ²Department of Animal Science, University of Connecticut, Storrs, CT.

Our objective was to determine the effects of water-soluble carbohydrates (WSC) and nonforage NDF inclusion within a physically adjusted NDF (paNDF) system on production, rumen fermentation, bacterial abundance, feeding behavior, and digestibility in late-lactation cows. Nine fistulated multiparous Holsteins (milk yield = 38 ± 4 kg; DIM = 130 ± 12 d; parity 2 or 3) were assigned to a triplicated 3×3 Latin square with 21-d periods (14 d adaptation, 7 d sampling). All 3 diets—control (CON), dextrose (DEX), and nonforage NDF (NFFS)—had similar CP

(16.5%), NEL (1.74 Mcal/kg DM), and forage NDF (19.1%). However, NFFS had higher nonforage NDF (13.3% vs. 9.3%) and lower starch (24.3% vs. 28%), whereas DEX had greater WSC (10.1% vs. 5.4%) but lower starch (24.5% vs. 28%) than CON. Diet and orts particle size were analyzed using the Penn State Particle Separator, with a sorting index (SI) of 100 indicating no sorting, values >100 indicating preference, and <100 indicating sorting against. Eating and rumination times were recorded via ear-attached accelerometers. The relative abundance (%) of ruminal bacterial species was measured using species-specific primers in qPCR. Fecal spot sampling (8 time points/cow) were used for total-tract digestibility estimation. Data were analyzed using a mixed-effects model, with period, Latin square, diet, and diet $\times$ period interaction as fixed effects, and cow as a random effect. DEX increased DMI by 6%–9% ($P = 0.04$) compared with CON and NFFS (30.0 vs. 27.9 kg/d). Cows on CON sorted against long particles (SI = 93.0), while DEX and NFFS sorted for them (SI = 110, $P < 0.01$). Milk yield and components did not differ ($P > 0.05$) across diets. Rumen pH, ammonia-N, and VFA were unaffected, except for increased iso-valerate (0.48% vs. 0.55%, $P = 0.03$) and a tendency for higher iso-butyrate ($P = 0.09$) in CON compared with DEX and NFFS. However, NFFS improved DM (63% vs. 70%; $P = 0.03$) and NDF (39% vs. 55%; $P < 0.01$) digestibility compared with CON and DEX. Additionally, NFFS tended to increase *Streptococcus bovis* ($P = 0.08$) and *Prevotella ruminicola* ($P = 0.09$) abundance. Whereas rumination time was unaffected, DEX tended to increase eating time by 9% compared with CON and NFFS. These findings suggest that WSC and nonforage NDF differentially influence intake, particle sorting, and fiber digestibility, but have minimal effects on production and rumen fermentation.

Key Words: nonforage fiber, particle size, feed particle sorting

2180 Effects of steeping grains and ration moisture on feed stability and productivity of lactating dairy cattle. L. Olthof^{*1}, L. Deters¹, D. Uyeh², D. Morris², and B. J. Bradford¹, ¹Department of Animal Science, Michigan State University, East Lansing, MI, ²Department of Biosystems and Agricultural Engineering, Michigan State University, East Lansing, MI.

Steeping grains before ration mixing may improve nutrient digestibility and productivity of cows; on the other hand, wetter diets are at greater risk of spoilage during warm weather. The objective of this study was to assess the effects of steeping grains vs. additional water at mixing time on feed stability, DMI, and productivity. Mid-lactation cows ($n = 26$) were enrolled in an incomplete replicated 3×3 Latin square design study conducted from June to August. Treatments were a control diet (CON; 48.8% DM), a diet with the same ingredients, with corn grain and soybean hull pellets steeped in water for 12 h (STP; 40.6% DM), and a diet with the same ingredients, with the same quantity of water added at mixing (WET; 40.6% DM). Organic acids (0.07% of DM) were added to all diets as a stabilizer. Cows were blocked by parity, DIM, and milk yield, then randomly assigned to treatment sequence within block. Each period lasted 21 d, with feed and milk (3 $\times$ /d) samples taken over the last 4 d of each period. Temperature of TMR piles (~ 50 kg as fed) was monitored continuously for 72 h ($n = 6$ /treatment). Mixed models were used to evaluate the fixed effect of treatment and random effects of period, block, and cow; when treatment was significant, means were separated by *t*-test. There was no spoilage observed among the diets fed, and feed stability did not differ among treatments. The STP diet decreased DMI compared with CON (1.3 ± 0.4 kg/d; $P = 0.001$) and to WET (0.8 ± 0.4 kg/d; $P = 0.05$). STP increased feed efficiency (ECM/DMI) compared with CON (0.05 ± 0.02 kg/kg; $P = 0.02$) and WET (0.04 ± 0.02 kg/kg; tendency $P = 0.08$). STP also decreased milk

protein concentration compared with CON ($0.05\% \pm 0.02\%$; $P < 0.01$) and WET ($0.05\% \pm 0.02\%$; $P = 0.02$) and tended to decrease protein yield compared with CON (0.05 ± 0.02 kg/d; $P = 0.08$). There were no significant effects on milk yield, ECM, milk fat or lactose concentration or yield, somatic cell score, or milk urea nitrogen. In conclusion, steeping grains—but not adding the same amount of water at mixing—decreased milk protein concentration and slightly increased feed efficiency in lactating dairy cows.

Key Words: compact total mixed ration, dry matter, efficiency

2181 Effects of corn grain processing on growth and carcass characteristics of beef-on-dairy steers and heifers. K. A. Juckem*, B. Halfman, C. Karls, D. M. Schaefer, R. Sterry, and L. F. Ferraretto, *University of Wisconsin, Madison, Madison, WI.*

The objective of this study was to evaluate the effects of corn grain processing on growth performance, fecal starch, liver abscesses, and carcass characteristics of Angus $\times$ Holstein cross cattle. Thirty steers, 30 heifers, and 2 dietary treatments were randomly assigned to 10 pens of 6 head each (same sex within a pen). The trial was 28 wk in duration, split into 2 periods. Treatments were unprocessed HMC (fed during period 1 and 2; $4,530 \pm 372$ μ m), or processed corn grain (dry, cracked corn fed during period 1; $2,449 \pm 507$ μ m, and rolled HMC during period 2; $3,008 \pm 257$ μ m) fed in growing (period 1, 46.1% starch, 23.1% aNDF) or finishing diets (period 2, 50.7% starch, 20.6% aNDF). Data were analyzed as a completely randomized design by period with initial body weight or intake at the beginning of period 1 used as covariate in a mixed model. The model included treatment, period, and their interaction as fixed effects, and pen(treatment) and sex as random effects. The diets with unprocessed corn grain had a 1.1 kg/d greater DMI ($P < 0.05$). There were no differences among treatments in weight, average daily gain, or carcass traits ($P > 0.10$). Therefore, the feed to gain ratio was 0.18 kg DMI/kg ADG lower ($P < 0.05$) for the processed corn grain diet. Fecal starch concentration was 8.8 percentage units greater ($P < 0.05$) for the unprocessed corn grain diet. There were only 2 liver abscesses ($n = 29$) in the unprocessed corn grain diet and no abscesses ($n = 30$) in the processed corn grain diet (data not analyzed statistically). These findings suggest that using processed corn grain in the diets of beef-on-dairy cattle may decrease feed to gain ratio and fecal starch by increasing starch digestibility compared with whole corn grain. There were also few instances of liver abscesses in both treatments.

Key Words: particle size, liver abscesses, fecal starch

2182 Evaluating the effects of a novel corn milling co-product on milk production, composition, and nitrogen and energy utilization in lactating dairy cows. C. M. Harris*, S. Tilton², A. L. Carroll¹, and P. J. Kononoff¹, ¹*Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE*, ²*The Andersons Inc., Maumee, OH.*

In 2023, the US produced over 28 million metric tons of distillers grains and solubles, with new methods of refining co-products emerging. One new corn milling co-product (50Y) is produced by mechanically separating fiber particles before fermentation, and the resulting product contains approximately 56% CP, 30% NDF, and 6.1% crude fat on a DM basis. The objective of this experiment was to determine the effects of the addition of 50Y to diets formulated to contain low concentrations of either RDP or RUP. In a twice replicated 5×5 Latin square design of 28 d periods, 10 multiparous Jersey cows were grouped by milk yield and randomly assigned 1 of 5 diets. The diets contained the following (DM basis): (1) 0% 50Y, 0% soybean meal (SBM), 13.3%

non-enzymatically browned soybean meal (NEBSM; Low RDP); (2) 13.3% 50Y, 0% SBM, 0% NEBSM (Low RDP + 50Y); (3) 0% 50Y, 8.62% SBM, 7.38% NEBSM (Low RUP); (4) 8.62% 50Y, 0% SBM, 7.38% NEBSM (Low RUP + 50Y); (5) 0% 50Y, 7.03% SBM, 9.51% NEBSM (positive control). Data were analyzed with PROC GLIMMIX in SAS (9.4). The model included fixed effect of treatment, period nested in square, and square and the random effect of cow nested in square. Significance was declared with a P -value ≤ 0.05 . The inclusion of 50Y did not affect dry matter intake, milk yield, fat percent, milk fat yield, or energy-corrected milk (19.2 ± 0.55 kg/d, 30.6 ± 0.71 kg/d, $5.00 \pm 0.113\%$, 1.52 ± 0.02 kg/d, and 37.9 ± 0.52 kg/d, respectively). Cows fed the Low RDP + 50Y diet produced milk with a higher protein percentage compared with those on the Low RDP diet, but milk protein yield was not different, averaging 1.08 ± 0.023 kg/d. No differences were observed in the concentrations of GE, DE, ME, or NEL, averaging 83.7 ± 1.94 , 54.7 ± 0.99 , 48.4 ± 0.46 , and 32.1 ± 0.53 Mcal/d, respectively. Results indicate that 50Y can be used to replace soy-based protein in dairy diets and maintain whole-animal energy utilization or milk production.

Key Words: high-protein corn milling co-product, milk production

2183 Effects of partial replacement of corn and oat silages with extracted stevia plant on production, behavior, and digestibility in dairy cows. M. Nehme Marinho*, Z. Sarwar¹, T. M. Adeoti¹, M. Perdomo¹, G. P. Marinho¹, A. C. S. Vincent¹, J. B. Cardoso¹, P. J. Kononoff², and J. E. P. Santos¹, ¹*University of Florida, Gainesville, FL*, ²*University of Nebraska, Lincoln, NE.*

Objectives were to evaluate the effects of replacing corn and oat silages with stevia plant on production, behavior, and total-tract (TT) apparent digestibility. Six primiparous and 12 multiparous cows (133 ± 16 d postpartum) were assigned to a sextuplicate 3×3 Latin square design with treatment sequence balanced for carry-over. Within parity, cows were ranked by ECM yield and assigned to one of 6 squares. Diets contained 60% forage and stevia replaced silages to represent 0% (ST0), 25% (ST25), or 40% (ST40) of diet DM. Each period lasted 21 d, with data collected from d 16 to 21. Intake of DM, BW, rumination, milk yield and composition were recorded daily. Fecal samples were collected from d 17 to 20, and feeding behavior evaluated every 5 min on d 16 and 21. Diet and fecal samples were analyzed for gross energy, and diet digestible energy (DE) was calculated. Data were analyzed by mixed-effects models with orthogonal polynomial contrasts evaluating linear (ST0 vs. $[\frac{2}{13}ST25 + \frac{11}{13}ST40]$) and quadratic (ST25 vs. $[\frac{3}{8}ST0 + \frac{5}{8}ST40]$) effects of proportion of stevia. Increasing dietary stevia increased DMI, time spent eating, and BW (667 vs. 675 vs. 672 ± 17 kg). However, increasing dietary stevia decreased milk yield, milk true protein content and yield, TT digestibility and rumination time, thus reducing diet DE content. Nevertheless, treatment did not affect the DE intake relative to milk NEL produced (Table 1). Replacing silages with stevia reduced feed conversion ratio, mainly because of a decrease in digestibility.

Key Words: coproduct, forage, stevia

2184 Performance of dairy cows fed distillers dried grains with solubles in replacement of soybean meal at constant protein and decreasing starch concentrations in the diet. D. E. Dorigo¹, J. T. R. Carvalho¹, W. R. Silva¹, A. J. C. Silva¹, J. O. Martins¹, G. H. O. Silva¹, M. N. Olimpio¹, S. F. Souza¹, R. B. Silva², C. D. S. Oliveira^{1,2}, R. A. N. Pereira³, and M. N. Pereira*, ¹*Universidade Federal de Lavras, Lavras, MG, Brazil*, ²*Better Nature Research Center, Lavras, MG,*

Table 1 (Abstr. 2183).

Item	Treatment			SEM	P-value	
	ST0	ST25	ST40		Linear	Quadratic
DMI, kg/d	25.2	28.6	29.5	0.4	<0.001	0.06
Time eating, min/d	289	296	314	12	0.01	0.30
Rumination, min/d	622	600	564	9	<0.001	0.09
Milk, kg/d	47.3	46.7	45.8	1.4	0.03	0.56
ECM, kg/d	48.5	47.3	46.4	1.0	0.002	0.80
ECM/DMI, kg/kg	1.94	1.67	1.58	0.03	<0.001	0.11
Fat, %	3.86	3.85	3.88	0.14	0.88	0.74
Fat, kg/d	1.81	1.78	1.77	0.04	0.18	0.89
Protein, %	2.97	2.84	2.74	0.07	<0.001	0.42
Protein, kg/d	1.40	1.32	1.25	0.03	<0.001	0.39
DM digestibility, %	62.6	56.9	52.2	1.3	<0.001	0.59
Diet DE, Mcal/kg	2.71	2.47	2.28	0.06	<0.001	0.67
DE intake/NEL milk, Mcal/Mcal	2.01	2.14	2.09	0.07	0.29	0.26

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Distillers dried grains with solubles (DDGS) is a corn ethanol coproduct with variation in nutrient composition due to source. The partial replacement of soybean meal (SBM) with DDGS is a strategy to formulate diets with more desirable AA profile, but it can affect diet fatty acids (FA) and NDF. This experiment evaluated the replacement of SBM with DDGS Inpasa (Sinop, Brazil). To maintain constant CP concentration in diet DM, SBM (50.3% CP) was mixed with finely ground mature corn (9.0% CP) to achieve the same CP content of DDGS (36.8% CP). Concentrations of CP (16.2% of DM), corn silage, whole cottonseeds, HMC and soyhulls were kept constant in all diets. Twenty Holstein cows (40.2 ± 4.6 kg/d, 154 ± 85 DIM, 607 ± 84 kg) were individually fed a sequence of 4 treatments (0%, 5%, 10%, and 15% DDGS in TMR DM) in 4 × 4 Latin squares, with 21-d periods (14-d adaptations). Diets contained (% of DM): 16.1, 12.6, 9.2, and 5.7 SBM and 7.0, 5.4, 3.7, and 2.1 ground corn. Starch concentrations were (% of DM): 25.6, 24.4, 23.2 and 22.0. Milk yield and composition and DMI were measured on d 15 to 21 of each period. Data were analyzed with MIXED of SAS and the model had the effects of square, cow(square), period and treatment. Treatment means were compared with Tukey ($P < 0.05$) when a $P < 0.10$ was detected for the treatment effect. Significant results ($P < 0.05$) will be reported. Cows fed 10% and 15% DDGS had higher DMI (27.0 kg/d) than 5% DDGS (25.7 kg/d), and cows fed 0% DDGS had the lowest DMI (24.8 kg/d). The yields of milk (39.5 kg/d) and ECM (34.6 kg/d) did not differ ($P > 0.42$). Feed efficiency (ECM/DMI) was lower with 10 and 15% DDGS than 0% and 5% DDGS (1.47 vs. 1.57). Milk fat concentration and yield were higher with 15% than 0% DDGS (3.32% and 1.270 kg/d vs. 3.24% and 1.227 kg/d). Preformed, stearic and oleic FA (g/100 g milk, g/d, g/100 g FA) were increased with more DDGS in the diet. Mixed and de novo FA did not differ ($P > 0.40$). Yields and concentrations of protein, casein and lactose did not differ ($P > 0.33$). The MUN was lower on 10% and 15% DDGS than on 0% and 5% DDGS (12.9 vs. 13.6 mg/dL). Cows fed 15% DDGS had higher milk fat yield and concentration, no difference in other milk components, and lower feed efficiency than 0% DDGS.

Key Words: distillers dried grains with solubles, corn coproduct

2185 Performance of dairy cows fed distillers dried grains with solubles in replacement of soybean meal at constant protein and

starch concentrations in the diet. J. T. R. Carvalho¹, D. E. Dorigo¹, A. J. C. Silva¹, M. A. Tiengo¹, J. O. Martins¹, R. B. Silva², C. D. S. Oliveira^{1,2}, R. A. N. Pereira³, and M. N. Pereira^{*1}, ¹Universidade Federal de Lavras, Lavras, MG, Brazil, ²Better Nature Research Center, Lavras, MG, Brazil, ³Empresa de Pesquisa Agropecuária de Minas Gerais, Lavras, MG, Brazil.

Distillers dried grains with solubles (DDGS) is a corn ethanol coproduct with variation in nutrient composition due to source. The partial replacement of soybean meal (SBM) with DDGS is a strategy to formulate diets with more desirable AA profile, but it can affect diet fatty acids (FA) and NDF. This experiment evaluated the replacement of SBM with DDGS Inpasa (Sinop, Brazil). To maintain constant CP concentration in diet DM, SBM (50.3% CP) was mixed with soyhulls (13.5% CP) to achieve the same CP content of DDGS (35.6% CP). Corn starch was kept constant at 20% of diet DM and CP was 16.2%. Twenty Holstein cows (37.6 ± 4.5 kg/d, 106 ± 66 DIM, 597 ± 63 kg) were individually fed a sequence of 4 treatments (0%, 5%, 10%, and 15% DDGS in TMR DM) in 4 × 4 Latin squares, with 21-d periods (14-d adaptations). Diets contained (% of DM): 14.2%, 11.2%, 8.3%, and 5.5% SBM and 16%, 14%, 12%, and 10% soyhulls. Data were analyzed with MIXED of SAS and the model had the effects of square, cow(square), period and treatment. Treatment means were compared with Tukey ($P < 0.05$) when a $P < 0.10$ was detected for the treatment effect. Significant results ($P < 0.05$) will be reported. Cows fed 5% DDGS had higher DMI than cows fed 0% and 15% DDGS (24.0 vs. 23.1 kg/d). The yields of milk (39.0 vs. 37.9 kg/d) and lactose were higher on 5 than 0% DDGS. Fat yield was higher on the 3 levels of DDGS than on 0% DDGS (1.372 vs. 1.311 kg/d). Preformed, stearic and oleic FA (g/100 g milk, g/d, g/100 g FA) were increased and Mixed was reduced with more DDGS in the diet. De Novo FA did not differ ($P > 0.18$). The yields of protein and casein were higher on 5% and 10% DDGS than on 0% DDGS. The concentrations of protein and casein were highest with 5% DDGS and there was no difference among the other 3 treatments. Cows fed the 3 levels of DDGS had higher yield of ECM than 0% DDGS (38.0 vs. 36.6 kg/d). The MUN was lower on 10% and 15% DDGS than on 0% and 5% DDGS (13.6 vs. 14.4 mg/dL). Feed efficiency measured as Milk/DMI and ECM/DMI did not differ ($P > 0.27$). The 5% DDGS diet was the most effective to increase DMI, milk yield and protein concentration relative to 0% DDGS.

Key Words: distillers dried grains with solubles, corn coproduct

2186 Effects of feeding alternative forage silages on early-lactation performance and gas production in multiparous Holstein cows. B. I. Dittrich^{*1,2}, E. A. French², E. M. Kammann², M. K. Breunig², K. F. Kalscheur², J. G. Franco^{2,3}, and H. M. White¹, ¹*University of Wisconsin–Madison, Madison, WI*, ²*US Dairy Forage Research Center, USDA-ARS, Madison, WI*, ³*Savanna Institute, Spring Green, WI*.

There are ecological and agronomic advantages to growing cover crops on dairy farms, especially if they can be fed as alternative forages. Our objective was to evaluate the effects of substituting alfalfa silage (AS) with various cover crop silages in early lactation of multiparous Holstein cows' diets on performance and gas production (GP). Cows (mean $\pm$ SD; 23 ± 3 DIM; $n = 80$) were enrolled in a randomized complete block design based on calving date (0 ± 2 WK), parity, total lifetime fat production, and ME305. Diets (TRT) were either 1) control (CTL; corn silage+AS), 2) rye (RYE), 3) rye+camelia+hairy vetch (R3X), 4) triticale (TRIT), or 5) triticale+camelia+hairy vetch (T3X) with each alternative silage replacing AS at 10% diet DM. Diets were formulated for 30% NDF, 18% CP, and 26% starch on DM basis (overall diet DM 44.5%) and fed for 10 weeks. Daily GP (CO_2 , O_2 , CH_4 , and H_2) were calculated from 10 spot samples within each time point collected (60 ± 7 and 90 ± 7 DIM) using GreenFeed units. Performance metrics (milk yield [MY], components, DMI) and daily averages of GP were analyzed using the lme function (R.4.4.2). All models included a fixed effect of TRT, WK, TRT $\times$ WK, random effect of cow within block, and WK as repeated measure. There were no TRT differences in DMI (27.4 kg/d) and GP (14,491, 10,140, 431, and 1.32 g/d for CO_2 , O_2 , CH_4 , and H_2). Average MY was greater for R3X vs. T3X (TRT 1 to 5; 50.1, 50.8, 52.4, 49.0, 46.5 kg/d; $P = 0.04$). Cows fed R3X vs. T3X also had greater milk lactose yield (TRT 1 to 5; 2.37, 2.40, 2.48, 2.31, and 2.19 kg/d; $P = 0.04$) and MUN (13.7, 14.0, 14.3, 13.4, and 13.2 mg/dL; $P = 0.05$), but lowest protein % (TRT 1 to 5; 2.96%, 2.93%, 2.92%, 3.02%, and 3.07%; $P = 0.02$). Cows fed R3X tended to have greater 4% FCM than T3X (TRT 1 to 5; 50.8, 53.4, 54.2, 51.9, and 48.6 kg/d; $P = 0.08$). Fat percentage differed by TRT $\times$ WK (TRT 1 to 5; 4.05%, 4.22%, 4.16%, 4.32%, 4.26%; $P = 0.03$) and fat yield tended to differ ($P = 0.08$) due to differences in lactation persistency. Cover crops can successfully be fed at 10% of diet DM in early lactation. Results demonstrate some differences in milk lactose, protein, and fat responses by TRT.

Key Words: cover crop, methane

2187 Reducing the forage content of the diet using the undigested forage-NDF: Effects on production, rumen environment, and digestibility. Y. Shaani^{*1,2}, J. Shpirer¹, T. Alon^{1,2}, L. Livshits¹, S. Mabjeesh², and U. Moallem¹, ¹*Department of Ruminant Science, Volcani Institute, Rishon LeZion, Israel*, ²*Department of Animal Science, the Hebrew University of Jerusalem, Rehovot, Israel*, ³*Department of Cattle Husbandry, Extension Service, Ministry of Agriculture, Rishon LeZion, Israel*.

Several factors influence the effectiveness of forage in ruminant rations: NDF content, the physical nature, fragility, digestibility, and so on. Recently, several studies suggested using the undigestible NDF fraction as a possible approach to achieve a more precise ration. The objectives were to reduce the forage content of the diet by maintaining the 48-h undigested forage NDF (uNDF48), and test the effects on production, rumen environment, and digestibility. 34 multiparous Israeli-Holstein dairy cows were divided into 2 groups according to milk yield, parity, DIM and BW, and fed in a crossover design study either 1) control (CTL) diet containing 35.8% forage (DM basis) and 10% forage uNDF48 based on wheat silage and wheat hay; 2) Low forage (LF)—diet containing

30.6% forage and 10.4% forage uNDF48 based on wheat silage and wheat straw. Diets were similar in CP (16.1%), NEL (1.73 Mcal), and starch (24.9%) content but differed in aNDF (34.5% for CTL vs. 36.7% for LF) content. Each period lasted 35 d, and data were collected during the last 21 d. Milk yields were recorded daily, and milk samples were taken weekly. At the fifth week of each period, 2 rumen samples were collected for VFA, pH, and ammonia 2 and 5 h after feeding, and 8 fecal samples were taken over a 48-h span for digestibility measurements. Production and efficiency data were analyzed as repeated measurements using the MIXED Procedure of SAS, and the effect of period and the interaction treatment $\times$ period were added to the model as fixed variables. The digestibility measurements were analyzed with the PROC GLM of SAS. Milk yield (52.2 and 51.7 kg/d, respectively; $P = 0.009$), 4% FCM yields (50.5 and 49.5, respectively; $P = 0.05$), and ECM yields (50.8 and 49.8 kg/d, respectively; $P = 0.01$) were higher in the CTL than in the LF. The milk fat and protein content were not different; milk fat yield in the CTL tended to be higher than in the LF diet (1.99 and 1.91 kg, respectively; $P = 0.06$), and milk protein yield was higher (1.67 and 1.63 kg, respectively; $P = 0.03$). The DMI was higher in the CTL than in the LF diet (32.7 and 31.7 kg/d, respectively), and the milk-to-DMI ratio was higher in the LF than in the CTL (1.59 and 1.57, respectively; $P < 0.001$). No differences were observed in the rumen pH, and ammonia and VFA concentration. The apparent total-tract digestibility of the CTL was higher than that of the LF diet ($P < 0.001$), respectively, for DM (62.6% and 56.8%), OM (65.1% and 59.6%), protein (61.9% and 53.8%), and aNDF (36.8% and 30.8%). In summary, reducing the forage content by balancing the diet for uNDF reduced the DMI, milk, fat, and protein yields and increased the efficiency of milk production; however, further research is required to fine-tune the proportion of forage uNDF and the optimal exchange of forage feedstuffs in the diet.

Key Words: forage, undigested forage NDF

2188 Effects of feeding diets containing a corn milling product on intake, milk production, methane production, digestibility, and rumination time in lactating Jersey cows. G. M. Fincham^{*1}, A. L. Carroll¹, H. C. Wilson², H. E. Ohlde², and P. J. Kononoff¹, ¹*Department of Animal Science, University of Nebraska–Lincoln, Lincoln, NE*, ²*OneTrak, Cargill Inc., Blair, NE*.

The objective of this study was to examine the effects of feeding a corn milling product (CMP; OneTrak, Cargill Inc.), in diets that varied the proportions of starch and forage NDF, on DMI, milk production, enteric methane production, digestibility, and rumination in lactating cows. Sixteen Jersey cows were arranged in a replicated 4×4 Latin square design consisting of 4 periods of 28 d. Cows were assigned to one of 4 treatments: CON (0% CMP); CMPb (Base CMP diet containing 40% CMP, 35% corn silage, 9.4% alfalfa hay, and 14.2% ground corn); CMPPhf (Base CMP diet with higher fiber by adding cottonseed and wheat straw, and reducing alfalfa hay and ground corn); CMPPhs (Base CMP diet with higher starch by reducing alfalfa hay and increasing ground corn). Data were analyzed with the GLIMMIX procedure in SAS (9.4) with fixed effects of treatment, square, and period nested in square and random effect of cow nested in square. Treatments did not affect DMI (19.8 ± 0.61 kg/d), milk yield (28.5 ± 1.16 kg/d), milk protein yield (0.95 ± 0.036 kg/d), or milk fat yield (1.37 ± 0.055 kg/d). Compared with CON, methane production decreased when cows consumed CMPb or CMPPhs (472.1, 424.0, 405.1 $\pm$ 17.52 L/d for CON, CMPb, and CMPPhs respectively), but cows consuming CMPPhf (445.8 $\pm$ 17.52 L/d) were similar to CON. Methane, per unit of ECM, decreased when cows consumed CMPb, CMPPhf, or CMPPhs (14.1, 12.6, 12.8, 12.2 ± 0.52 L/kg for CON, CMPb, CMPPhf, and CMPPhs respectively). Digestibility of

aNDFom increased when cows consumed CMPb, CMPhf, or CMPhs (43.2%, 49.7%, 48.6%, 48.7% $\pm$ 1.07% for CON, CMPb, CMPhf, and CMPhs respectively). Compared with CON (360.3 $\pm$ 19.10 min), diets containing CMP were not different in rumination activity. Among diets containing CMP, cows consuming CMPhf spent significantly more time ruminating (403.4, 355.1, 340.0 $\pm$ 19.10 min for CMPhf, CMPb, and CMPhs respectively). Results of this study indicate that feeding CMP to Jersey cows does not affect DMI, milk yield, or milk composition, but enteric methane production may be reduced when cows consume CMP.

Key Words: corn milling product, methane, rumination

2189 Effects of determining NDF with or without an acetone wash on nutrient digestibility in cows fed fat-supplemented diets.

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Previously, we observed bioequivalence in determining NDF and iNDF content of feeds with or without an acetone wash step (WS). The objective of this study was to evaluate the effects of WS on nutrient digestibility in lactating dairy cows fed fatty acid (FA)-supplemented diets. iNDF was used as an internal marker to predict fecal output to determine apparent total-tract digestibility. NDF and iNDF analyses of feeds, Orts, and feces were performed with a WS using either water and acetone (AC) or water only (WO). Thirty-six multiparous Holstein cows (146 $\pm$ 55 DIM) were used in a truncated Latin square with 2 consecutive

35-d periods. Treatments were diets containing 1) no supplemental FA (control; CON); 2) a C16:0-supplement (PA, 84% C16:0, 4% C18:0, and 9% C18:1); and 3) a C16:0+C18:0-supplement (MIX, 33% C16:0, 53% C18:0, and 5% C18:1). Supplements were fed at 1.5% DM and replaced soyhulls in CON. Statistical analysis considered the random effect of cow nested in square and the fixed effects of WS, treatment, period, square, and WS $\times$ treatment interaction. Contrasts evaluated the overall effect of FA supplementation [CON vs. FA; 1/2 (PA + MIX)] and PA vs. MIX. We observed no interactions between WS and treatment for nutrient digestibility (all $P > 0.75$), indicating that the effect of FA supplementation on nutrient digestibility is independent of WS used during NDF and iNDF analysis. Compared with AC, WO decreased NDF digestibility (2.4%, $P = 0.01$) but had no effect on the digestibility (all $P > 0.14$) or absorption (all $P > 0.30$) of 16-carbon, 18-carbon, and total FA. Compared with CON, FA supplementation had no effect on NDF digestibility ($P = 0.13$) but decreased the digestibility of 16-carbon (4.6%), 18-carbon (3%), and total FA (3.9%, all $P < 0.01$). Compared with MIX, PA increased NDF digestibility (3.1%, $P = 0.02$), had no effect on the digestibility of 16-carbon FA ($P = 0.39$), and increased the digestibility of 18-carbon (12%) and total FA (6.4%, both $P < 0.01$). Overall, a C16:0-enriched supplement improved NDF and total FA digestibility compared with a C16:0+C18:0-enriched supplement. In conclusion, irrespective of acetone use in the WS for NDF and iNDF analysis there were no interactions between WS and treatments for nutrient digestibility.

Key Words: NDF analysis, palmitic acid, stearic acid

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2190 Effect of a combination of dietary palmitic and oleic acid supplements on animal performance and body weight in lactating dairy cows. J. P. Marden*, J. M. Baetjer, and C. Cordts, *Berg + Schmidt GmbH & Co. KG Animal Nutrition, Hamburg, Germany.*

Increasing dietary oleic acid (OA—*cis* C18:1) content of lactating cow diets in combination with palmitic acid (PA—C16:0) is a common practice to boost milk performance, increase nutrient digestibility and improve energy balance. However, as unsaturated fats disturb ruminal fermentation, care should be taken in well balancing fatty acids for optimum use. The objectives of this study were to compare the supplementation of a high PA diet (fat content = 99%) versus a prilled form of $\geq 60\%$ PA + $\geq 23\%$ OA (fat content $\geq 97\%$) and to observe their effects on milk production, fat, protein contents and body weight of lactating dairy cows. A total of 171 cows (mean DIM = 184) were enrolled in a complete randomized design of 20 weeks of supplementation. Cows were divided into 2 groups according to milk yield, days in milk and body weight. They were assigned to a PA (n = 84) and PA + OA diet (n = 87). The basal diet was mainly composed of corn silage, ground corn, alfalfa (62%) with the remaining being concentrates and minerals (38%). Both groups were supplemented with 0.47 kg/h per day of fat supplement in the basal diet. Milk yield, protein, fat and body weight were recorded daily over the trial period. Data were analyzed using univariate model of SPSS considering the fixed effect of treatment and random effect of the animal. Results showed that PA + OA group yielded significantly ($P < 0.05$) higher milk production (45.8 kg/h per day) compared with PA group (45.2 kg/h per day). Energy corrected milk was significantly ($P < 0.05$) higher in early lactating animals (DIM < 100) than in mid/late lactation for PA + OA treatment (+0.85 vs. +0.64 kg/h per day, respectively) when compared with PA. Although the animals increased their performance, PA + OA fed cows showed no loss of weight ($P < 0.05$) compared with PA group. A 5.1-kg increase of BW per animal was recorded during the whole trial. In conclusion, the reported data suggested that combining PA + OA favored animal performance without affecting body condition. It can be a suitable one product-mix to counteract milk depression in early lactation cows.

Key Words: fatty acid profile, body weight, milk yield

2191 Decreasing the risk of milk fat depression by feeding a diet with reduced dietary cation-anion difference in lactating cows. H. Hu*, J. Kim¹, K. Park¹, Y. A. Ben Meir², and C. Lee¹, ¹*The Ohio State University, Wooster, OH*, ²*Institute of Animal Science, Agricultural Research Organization, Rishon LeZion, Israel.*

Previously, a diet that was intentionally reduced for dietary cation and anion difference (DCAD) was examined to lower urine pH and decrease ammonia emission. However, feeding a diet with reduced DCAD negatively affected production, especially milk fat. This experiment was aimed to evaluate the effects of supplementing a reduced DCAD diet with additives that might alleviate milk fat decreases on milk production and nutrient digestibility. Sixteen mid-lactation Holstein cows were assigned to a replicated 4 × 4 Latin square design with the following dietary treatments: a control diet (CON; 209 mEq/kg DM of DCAD); the CON diet with reduced DCAD (rDCAD; 10 mEq/kg DM); the DCAD diet supplemented with either palmitic acid (Palm) or 2-hydroxy-4-methylthio butanoic acid (HMTBa). Each period included 14 d of diet adaptation and 7 d of sampling. Spot fecal samples were collected from 2 blocks of cows (6 time points over 3 d to represent

every 4 h sampling in a feeding cycle; i.e., n = 8/treatment). Indigestible NDF was used as a marker to estimate fecal outputs and calculate nutrient digestibility. Data were analyzed using the MIXED procedure of SAS where cow within square and period were random effects and treatment was a fixed effect. Dry matter intake was lower ($P = 0.01$) for PALM and HMTBa compared with CON. Milk yield decreased for rDCAD, Palm, and HMTBa (36.8 vs. 38.6 kg/d; $P < 0.01$) compared with CON. Milk fat yield was lower (1.57 vs. 1.69 kg/d; $P = 0.05$) for rDCAD compared CO, but similar milk fat yields were observed among CON, Palm, and HMTBa. Similarly, ECM was lower (41.9 vs. 44.3 kg/d; $P = 0.05$) for rDCAD compared with CON, but a difference was not observed among CON, Palm, and HMTBa. Total-tract OM, N, and NDF digestibility did not differ among treatments. In conclusion, reducing DCAD decreased DMI and ECM and confirms the importance of DCAD for optimal production. However, supplementation of Palm or HMTBa would be an effective strategy to avoid a decrease in milk fat when DCAD is reduced in certain conditions (e.g., inclusion of corn distillers grains).

Key Words: 2-hydroxy-4-methylthio butanoic acid, digestibility, palmitic acid

2192 Effects of fat supplements on total de novo synthesized fatty acids using natural stable carbon isotope enrichment differences of feed sources. Y. Adeniji* and K. J. Harvatine, *Department of Animal Science, The Pennsylvania State University, University Park, PA.*

All the <16C fatty acids (FA) in milk fat originate from de novo synthesis. However, a portion of the 16C FA are also from de novo synthesis but is not discernable in normal experiments. We hypothesize that the de novo portion of 16C FA would be decreased with increasing dietary FA and that the response would differ between 16:0 and 18:0 supplements. The objective was to quantify the de novo portion of milk 16 and 18C FA. Natural ¹³C isotope enrichment of individual FA in feeds and milk FA from a previous experiment with 12 mid lactating Holstein cows in a 4 × 4 Latin square study with 21-d periods was determined using a GC isotope ratio mass spectrometer. Treatments were a low-fat diet with ~80% corn ingredient (CON) and supplementation with a high palmitic (HP; 91% 16:0), high stearic (HS; 92.6% 18:0), or palmitic-stearic blend (PS; 45.3% 16:0 and 49.1% 18:0) at 1.95% of diet DM. Data were analyzed using a mixed model in JMP Pro 18, with cow and period as random effects and treatment as a fixed effect. Increasing dietary FA had no effect on the $\delta^{13}\text{C}$ of <16C FA. The de novo portion of 16C FA in the control was calculated to be 86% using a mixture model based on the enrichment of <16C FA and 16C in the basal diet. The de novo portion of 16C FA in the FA treatment were calculated based on enrichment of the <16C FA and the supplement assuming equal transfer of the basal diet. The de novo portion of 16C decreased to 82.2% in HS, 64.2% in PS, and 47.6% in HP ($P < 0.001$). However, total grams of de novo 16C FA was similar for CON, HS, and PS, but was 81 g/d lower in the HP compared with CON ($P < 0.001$). Transfer efficiency of the supplemented 16C increased with increasing PA in the supplement (40.3% in HP, 32.9% in PS, and 8.06% in HS; $P < 0.001$). Supplement contribution to milk 18C FA was highest in HS and lowest in HP (125 g/d vs. 27.6 g/d; $P < 0.001$). Overall, total de novo FA synthesis was similar for CON, PS, and HS but was decreased by 125 g/d in HP compared with CON. In conclusion, transfer of FA into milk FA

declined with increasing exogenous supply and palmitic acid reduced de novo synthesis of 16C FA.

Key Words: transfer efficiency, modeling, milk fat

2193 Comparison of analysis of milk fatty acid profile by gas chromatography or mid-infrared spectroscopy. J. P. A. Rezende¹, L. C. B. Pimenta¹, M. C. S. Santos¹, J. Petrini², and M. A. C. Danes^{*1}, ¹Universidade Federal de Lavras, Lavras, MG, Brazil, ²Clinica do Leite Ltda, Piracicaba, SP, Brazil.

The milk fatty acid (FA) profile is frequently used as a tool for assessing the nutritional status of lactating cows, especially with the advent of mid-infrared (MIR) spectroscopy calibration curves for its measurement. This study evaluated the precision and accuracy of MIR curves used by a Brazilian laboratory (Clínica do Leite Ltda) for measuring de novo (sum of C4:0, 6:0, 8:0, 10:0, 12:0, 14:0, and 14:1) and preformed (sum of C15:0, 17:0, 18:0, 18:1, 18:2, 18:3, 20:0, 20:2, 22:0, 24:0) FA in milk. Milk samples were collected from 21 Holstein cows (milk yield = 34.6 kg/d, fat = 3.49%) at 3 time points, every 21 d, totaling 63 samples, which were analyzed by MIR and GC. The samples for MIR analysis were composed of milk from all 3 milkings of the day, preserved with bronopol and analyzed within a maximum of 3 d. For GC, a 500-mL milk aliquot was collected only from the first milking of the day, frozen, and stored at -20°C until analysis. The results of the MIR were compared with the GC analyses calculating the linear equation, intercept bias, slope bias, coefficient of determination (R²), and the concordance correlation coefficient (CCC), using R Studio. On average ± SD, MIR analysis resulted in 0.74 ± 0.12 de novo, 1.31 ± 0.19 preformed as percentage of milk, and 20.7 ± 1.67 de novo, 36.9 ± 3.0 as percentage of FA. Analysis with GC resulted in 0.82 ± 0.13 de novo, 1.47 ± 0.22 preformed as percentage of milk, and 23.2 ± 1.7 de novo, 41.4 ± 2.9 preformed as percentage of FA. Differences in milk collection may have influenced the intercept bias, affecting the accuracy. The best results were observed when expressing FA as percentage of milk, with high R² and CCC and low slope bias, indicating good precision for use in the field (Table 1).

Key Words: nutritional monitoring, precision nutrition

2194 Effects of n-3 fatty acid supplementation during the transition period on uterine involution. H. L. Reisinger^{*1}, M. L. Miller¹, A. Santos¹, T. Minela¹, J. R. Pursley¹, G. A. Contreras², A. L. Lock¹, and B. J. Bradford¹, ¹Department of Animal Science, Michigan State University, East Lansing, MI, ²Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI.

Table 1 (Abstr. 2193). Agreement and bias assessment of linear regression between milk de novo and preformed fatty acid (FA) estimation by mid-infrared spectroscopy or GC

Item	Linear equation	Intercept bias	Slope bias	R ²	CCC
De novo, % of milk	y = 0.088 + 0.998x	0.088	-0.001	0.87	0.74
De novo, % of FA	y = 7.399 + 0.761x	7.399	-0.239	0.54	0.36
Preformed, % of milk	y = 0.017 + 1.112x	0.0168	0.111	0.88	0.71
Preformed, % of FA	y = 14.714 + 0.723x	14.714	-0.277	0.58	0.35

Omega-3 (n-3) fatty acids (FA) are reported to improve fertility, but the mechanism is unknown. One possibility is through improved uterine health in the postpartum period. Therefore, we evaluated the effects of n-3 FA supplementation throughout the transition period on uterine involution. Multiparous Holstein cows (n = 35) were enrolled in a randomized incomplete block design. Treatments were a control diet containing a calcium salt of palm oil at 0.60% diet DM (CON) or a diet containing a calcium salt enriched in eicosapentaenoic acid (EPA; C20:5) and docosahexaenoic acid (DHA; C22:6) at 0.90% diet DM (N3). Diets were balanced for dietary supply of palmitic, oleic, and linoleic acids. Treatment diets were fed 21 d before expected calving through 23 DIM. All cows were fed a common diet after 23 DIM. Uterine horns were scanned with ultrasound weekly from 1 to 8 wk after calving. The cross-section diameter of each uterine horn was measured by digital calipers and horn area was calculated. Fluid area within the horns was calculated separately. Data were analyzed using the random effect of block and cow nested within block, and fixed effects included treatment, time, parity, and their interactions. N3 did not affect the amount of fluid within the horns (*P* > 0.69). Treatment did not have an overall effect on previously gravid horn area, but there was a treatment by time interaction (*P* = 0.07), with N3 decreasing horn area in wk 5 (331 ± 29 mm² CON vs. 221 ± 19 mm² N3; *P* < 0.01). N3 significantly decreased previously nongravid horn area across the 8 wk (255 ± 12 mm² CON vs. 220 ± 10 mm² N3; *P* = 0.03). There was also a treatment by time interaction (*P* = 0.07), where N3 decreased area of the previously nongravid horn in wk 5 (*P* < 0.01). Across treatments, the previously nongravid horn area was smaller in second vs. third+ parity cows (218 ± 11 mm² vs. 257 ± 10 mm²; *P* = 0.02). This effect was not observed in the previously gravid horn. Overall, N3 appeared to accelerate uterine horn involution, suggesting a potential role for n-3 FA in promoting uterine health postpartum.

Key Words: uterine regression, docosahexaenoic acid, reproduction

2195 Lysolecithin addition to ruminant feed: Mode of action insights. V. Van Hoeck, I. Somers, R. Spaepen, B. Vennekens, E. E. Gültepe*, and B. Forier, Kemin Europa NV, Herentals, Belgium.

Lysolecithins (LL) are world-widely recognized for aiding nutrient digestion and absorption of monogastric animals, with recent evidence suggesting benefits in ruminant diet. A lot of theories have been put forward to explain these benefits in ruminants, but, up till now, there is no scientific “mode-of-action” evidence available. To bridge this gap, a LL-based product (soybean LL with 11% lysophospholipids; Kemin Animal Nutrition and Health, Belgium) was incubated for various durations in an in vitro rumen batch system. The rumen fluid/buffer ratio was set at 1:4, and the inoculum was obtained from 3 rumen-fistulated dairy cows on a basal lactation diet. Analyses included gas chromatography, p-NMR, and 16S metagenomics sequencing, assessing VFA production, phospholipid characterization, and rumen microbiome. There was a control treatment and a LL treatment. For the latter treatment, 2.5 g of test product (soybean LL) was added per 500 mL. Each treatment had 6 replicates. Statistical procedures were carried out in JMP, V15.0.0. The effect of treatments on variable parameters was analyzed with ANOVA. Post hoc comparisons between treatments were investigated by Duncan’s test. Results showed that 73.4% of the total LL remained intact after 4 h, 43.6% after 8 h, and 8.4% after 24 h of rumen incubation. Total VFA production tended to be higher in treated flasks compared with control at all incubation times (29.1%, 24.7%, 12.5% higher at 4 h, 8 h, and 24 h, respectively with *P* < 0.1). The effect seen on increased propionate, caproate and butyrate production was significant (*P* < 0.05). Absolute methane production was unaffected, whereas ratio of methane:VFA

decreased with treatment ($P = 0.01$). Abundances of *Ruminococcaceae*, *Desulfovibrionales*, and *Cyanobacteria* were significantly increased by treatment ($P < 0.05$), suggesting enhanced fiber, sulfur, and nitrogen utilization. Microbial diversity (based on number of ASV) was also higher in the treatment compared with the control group. Overall, the data reveal that soy-bean based LL in the in vitro fermentation model itself could increase VFA production, decrease methane: VFA production ratio and alter the in vitro rumen microbiome diversity. These effects could potentially lead to better animal performance and better production of milk components.

Key Words: lysolecithin, rumen fermentation, microbiome diversity

2196 Raw and roasted high oleic soybeans increase oleic acid content of plasma lipids in high-producing dairy cows. A. M. Bales, K. M. Gouveia*, and A. L. Lock, *Michigan State University, East Lansing, MI*.

We determined the effect of feeding raw or roasted high oleic acid soybeans (HOSB) on plasma fatty acid (FA) profiles of dairy cows. Thirty-six multiparous Holstein cows (45.6 ± 6.22 kg/d of milk; 110 ± 61 DIM) were randomly assigned to treatment sequences in a 4×2 Truncated Latin square design with 35-d periods. Treatments were: control (CON), 16% roasted HOSB (RST), 16% raw HOSB (RAW-D), and 16% raw HOSB + by-pass protein (RAW-U). HOSB (17.5% DM total FA; 83% oleic acid) replaced conventional soybean meal and hulls in treatment diets and heat-treated by-pass protein replaced soybean meal in RAW-U to maintain diet nutrient composition (% DM) of 21% forage NDF, 28% starch, and 17% CP. Total dietary FA content was 2.8%, 4.9%, 5.1%, and 5.1% DM, respectively. The statistical model included the random effect of cow within square and fixed effects of square, treatment, period, and their interactions. Pre-planned contrasts were the overall effect of HOSB {CON vs. SOY [1/3 (RST + RAW-D + RAW-U)]}, effect of roasted HOSB {RST vs. RAW [1/2 (RAW-D + RAW-U)]}, and effect of protein source (RAW-D vs. RAW-U). Order of results are: CON, RST, RAW-D, and RAW-U with plasma lipid results reported as g/100 g total FA. Compared with CON, SOY increased stearic acid (C18:0; 15.7, 16.2, 17.1, 17.2; $P < 0.01$), oleic acid (C18:1n-9; 4.98, 11.8, 12.2, 11.9; $P < 0.001$), and linolenic acid (C18:3n-3; 2.97, 3.37, 3.68, 3.63; $P < 0.001$) and decreased (both $P < 0.001$) palmitic acid (C16:0; 9.75, 8.80, 8.67, 8.71) and linoleic acid (C18:2n-6; 49.1, 44.8, 43.1, 43.3). Compared with RAW, RST increased *trans*-18:1 biohydrogenation intermediates ($P < 0.01$) and C18:2n-6 ($P = 0.01$), decreased C18:0 and C18:3n-3 (both $P = 0.01$), but did not affect C16:0 or C18:1n-9 (both $P > 0.30$). There were no differences between RAW-D and RAW-U (all $P > 0.40$). Overall, HOSB inclusion at 16% DM altered plasma lipid profiles, increasing oleic and linolenic acids and decreasing linoleic acid content. Our results suggest that roasting HOSB may affect the availability and biohydrogenation of FA in the rumen, indicated by differences in C18:0 and *trans*-18:1 compared with raw HOSB.

Key Words: oleic acid, high oleic soybean, fatty acid

2197 Effects of varying degrees of fatty acid saturation on milk production and greenhouse gas emissions of Holstein dairy cows. E. Sarmikasoglou, R. E. Barber*, A. L. Lock, and M. J. VandeHaar, *Michigan State University, East Lansing, MI*.

Dietary fats decrease CH₄ emissions, but effects of different fatty acids (FA) are not clear. Our objective was to determine effects of FA supplementation with 3 varying degrees of saturation on DMI, milk production, and CH₄ of Holstein cows. Twenty-four primiparous, and

12 multiparous Holstein cows (130 ± 37 DIM, 38 ± 6.1 kg milk/d, 613 ± 62 kg BW) were enrolled in a 4×2 truncated Latin square design with two 21-d experimental periods, balanced for carryover effects. Treatments were control (CON, diet without supplemental FA), and 3 diets supplemented with FA blends that were enriched in SFA, MUFA, or PUFA. The control diet contained 35% corn silage, 24% alfalfa silage, 11% corn grain, and 22% other concentrates and was formulated to 30% NDF, 18% CP, and 25% starch and fed as TMR. The FA supplemented diets were iso-energetic and contained various supplemental FA sources in place of corn and soyhulls so that all FA supplemented diets contained 1.0% (% diet DM) added C16:0 and 1.0% C18 or greater FA. These longer FA were 0.83% C18:0 and 0.14% C18:1 for SFA; 0.08% C18:0, 0.74% C18:1, and 0.16% C18:2 for MUFA; and 0.12% C18:0, 0.34% C18:1, 0.33% C18:2, 0.02% C18:3, and 0.09% EPA+DHA for PUFA. Data were analyzed with PROC GLIMMIX of SAS 9.4 and preplanned contrasts of 1) CON vs. FA treatments; 2) SFA vs. UFA (MUFA and PUFA); and 3) MUFA vs. PUFA. No parity $\times$ treatment interactions were observed. Supplemental FA did not alter DMI or milk production ($P > 0.20$). Saturated FA increased DMI, BW gain, and yields of ECM, fat, protein, and lactose compared with UFA ($P < 0.05$), but PUFA vs. MUFA were not different (all $P > 0.10$). Compared with CON, FA supplements did not alter CH₄ output, yield, or intensity but reduced CO₂ output. Compared with SFA, UFA decreased CO₂, CH₄, and H₂ but not CH₄ yield or intensity. No differences in gasses were observed for MUFA vs. PUFA. In conclusion, FA supplementation had no overall effect on production or methane output, yield or intensity, and SFA increased intake, milk production and CH₄ emissions with no effect on CH₄ intensity relative to the other treatments.

Key Words: fatty acid, methane

2198 Effects of drying method on fatty acid profile of fecal samples for digestion studies. S. Burnett*, M. Husnain, and K. J. Harvattine, *Penn State University, State College, PA*.

Exposure to oxygen and higher temperatures increases the risk of fatty acid (FA) oxidation. The effect of oven drying compared with freeze drying has been reported for feeds but has not been explored for fecal samples which are much higher in free FA that increases the risk of oxidation. Our objective was to compare the FA profile of fecal samples dried in a freeze drier or a forced air oven at 2 temperatures. We hypothesized that oven drying would decrease the concentration of unsaturated FA. Fecal samples were collected from 8 primiparous Holstein cows (>80 DIM) during a study feeding low- and high-fat diets in a 4×4 Latin square design. Fecal samples were aliquoted into 3 samples per cow and stored at -20°C until drying. The drying methods were 1) freeze-drying (FD); 2) forced-air oven at 105°C for 8 h (O105) and 3) forced-air oven at 55°C for 48 h (O55). Data were analyzed in JMP Pro 18. The model included the random effect of sample and fixed effect of drying method. Both oven-drying methods decreased the concentration of 18:2 *cis*-9, *cis*-12 (11.0% vs. 5.97% and 3.70%; $P < 0.01$) and 18:3 *cis*-9, *cis*-12, *cis*-15 (1.66% vs. 0.85% and 0.63%; $P < 0.01$). Due to this loss, oven-dried samples were higher in 18:0, particularly for O55 compared with FD (47.6% and 41.2%; $P = 0.02$). There was no difference in concentrations of 16:0 or 18:1 *cis*-9 between drying methods. Overall, total 18C concentrations tended to be higher in freeze-dried samples compared with both oven-drying methods (68.3% vs. 65.3% and 64.5%; $P = 0.08$). Similar differences were observed in a second data set with oven-dried samples stored for a longer duration compared with freeze-dried samples. Overall, drying method influenced the FA profile of fecal samples by decreasing the concentration of unsaturated

FA and increasing the concentration of saturated FA which may influence digestibility calculations.

Key Words: palmitic acid, stearic acid, unsaturated fatty acid

2199 Effects of dietary palmitic and calcium-salt lipid supplements on milk fatty acid composition in Holstein dairy cows. F. A. Gutierrez-Oviedo^{*1}, M. J. Farricker¹, V. Ramos¹, M. You¹, A. Javaid¹, V. R. Basnayake¹, N. D. Seneviratne¹, D. C. Reyes¹, J. R. Newbold², R. M. Kirkland³, and J. W. McFadden¹, ¹*Department of Animal Science, Cornell University, Ithaca, NY*, ²*Dairy Research Centre, SRUC, Dumfries, UK*, ³*Volac Wilmar Feed Ingredients Limited, Orwell, Hertfordshire, UK*.

Our aim was to evaluate the effects of a palmitic acid supplement vs. calcium salts supplements on milk fatty acid composition and yield in Holstein cows. Thirty-six multiparous Holstein cows (138 ± 13 DIM, 43 ± 6.4 kg of milk/d) were enrolled in a split-plot design. The main plots were HP (88.7% PA) or PO (46.8% PA and 35.8% OA). Within each plot, cows were assigned to one of 3 fatty acid (FA) levels (0%, 1.5%, or 3% of diet DM) in a 3×3 Latin square with three 21-d periods. Cows were fed a corn silage-based diet (14.3% CP; 31.4% aNDFom; 24% starch), where dietary FA replaced fiber. Data were analyzed using a mixed model with fixed effects of plot, FA level, period, square, and plot $\times$ FA level, with cow nested within square as a random effect. The main plot effect was tested using Cow (Square) as the error term. Preplanned contrasts compared HP and PO at 1.5% and 3.0% FA levels. Milk samples (d 19–21) were analyzed by gas chromatography. Palmitic acid concentrations (g/100 g fat) increased with HP but decreased with PO ($P < 0.01$). Stearic acid concentrations were higher with PO than HP ($P < 0.01$). Oleic and *cis*-9, *trans*-11 CLA concentrations increased with PO supplementation ($P < 0.01$). De novo FA concentrations declined with increasing FA levels ($P < 0.05$). Mixed FA concentrations were greater with HP and lower with PO ($P < 0.01$), while preformed FA concentrations decreased with HP and increased with PO ($P < 0.01$). Saturated FA concentrations were unaffected by HP but decreased with PO ($P < 0.01$). The MUFA, PUFA, and total UFA concentrations increased with PO ($P < 0.01$). Palmitic acid yields were higher with HP than PO ($P < 0.01$). Stearic acid yields were higher with PO than HP ($P < 0.01$). Oleic acid and CLA yields increased with PO ($P < 0.01$). De novo FA yields did not differ between treatments ($P = 0.12$). Mixed FA yields were greater with HP, relative to PO ($P < 0.01$), whereas preformed FA yields were greater with PO ($P < 0.01$). Yields of SFA were not affected by FA product ($P = 0.59$). To conclude, lipid supplementation type and level distinctly modify FA composition and yields.

Key Words: calcium-salt, palmitic acid, milk fatty acid

2200 A meta-analysis examining the effect of fatty acid blends on nutrient digestibility and production responses of dairy cows. J. M. dos Santos Neto^{*1}, J. de Souza², and A. L. Lock¹, ¹*Michigan State University, East Lansing, MI*, ²*Perdue AgriBusiness, Salisbury, MD*.

Our objective was to evaluate the effects of supplementing fatty acid (FA) blends of C16:0+C18:1 and C16:0+C18:0 using a meta-analytical approach on nutrient digestibility and production responses of dairy cows. Our data set included 11 studies from Michigan State University. We compared 3 dietary treatments: 1) a control diet without FA supplementation (CON, $n = 170$), 2) a diet supplemented with a FA blend containing ~70% C16:0 and ~20% C18:1 (PAOA, $n = 492$), and 3) a diet supplemented with a FA blend containing ~46% C16:0 and ~37% C18:0 (PASA, $n = 96$). The FA blends (mean $\pm$ SD) were included at 1.63

$\pm 0.18\%$ of diet DM in PAOA and $1.51 \pm 0.07\%$ of diet DM in PASA. The FA blends were prepared using different proportions of C16:0-enriched supplements, C18:0-enriched supplements, and calcium salts of palm FA. Our statistical model included the fixed effect of treatment and the random effects of study, period within study, and cow within study. Days in milk was used as a covariate. Contrasts evaluated the overall effect of FA supplementation (FAT) [CON vs. FA; 1/2 (PAOA + PASA)] and PAOA vs. PASA. Compared with CON, FAT had no effect on DMI ($P = 0.95$) or NDF digestibility ($P = 0.75$) and decreased (all percentage units) 16-carbon (4.58%, $P < 0.01$), 18-carbon (1.56%, $P = 0.01$), and total FA digestibility (2.95%, $P < 0.01$). Compared with CON, FAT had no effect on milk protein yield ($P = 0.71$) and increased the yields of milk (1.10 kg/d, $P = 0.03$), milk fat (0.10 kg/d), 3.5% FCM (2.23 kg/d), and ECM (1.97 kg/d, all $P < 0.01$). Compared with PASA, PAOA had no effect on DMI ($P = 0.19$), and increased the digestibility (all percentage units) of NDF (2.38%), 16-carbon (6.00%), 18-carbon (9.51%), and total FA (7.72%, all $P < 0.01$). Compared with PASA, PAOA had no effect on the yields of milk ($P = 0.45$) or milk protein ($P = 0.99$), increased milk fat yield (0.07 kg/d, $P = 0.05$), and tended to increase 3.5% FCM (1.45 kg/d, $P = 0.06$) and ECM (1.22 kg/d, $P = 0.08$). Overall, FA supplementation increased the yields of milk and milk fat without affecting DMI. However, a FA blend of C16:0+C18:1 improved nutrient digestibility and increased milk fat yield compared with a FA blend of C16:0+C18:0.

Key Words: oleic acid, palmitic acid, stearic acid

2201 Effects of oleic acid concentration in milk fat on the thermal properties of butter oil. M. Husnain^{*1}, I. Salfer², and K. J. Harvatine¹, ¹*The Pennsylvania State University, State College, PA*, ²*University of Minnesota, Minneapolis, MN*.

The melting properties of butter affects its spreadability at room temperature and is an important characteristic to some consumers. Melting properties are influenced by the fatty acid profile with previous work identifying key influences of palmitic (PA) and oleic acid (OA). Our objective was to characterize the effect of OA concentration on solid fat content (SFC) at room temperature across a broader range. Milk fat samples were selected from a previous experiment that abomasally infused 350 g/d of a free fatty acid stock that was 79.6% OA either over 22 h/d or 8 h during the daytime or nighttime. Cows were also milked 4 times per day and samples from each milking from 5 cows were selected to provide a broad uniform distribution of OA concentration for regression analysis. Butter oil was prepared by high-speed centrifugation and solid fat content at 20°C observed by differential scanning calorimetry (DSC). Milk fat OA ranged from 15.4% to 34.6% and PA ranged from 16.9% to 29.5% of FA. The relationship between melting properties and selected FA was first analyzed by bivariate regression and second by multivariate analysis using JMP Pro 18. There was a strong negative linear relationship between SFC at 20°C and OA as a percentage of FA (intercept = 42.9 and slope = -0.998 ; $P < 0.001$, $R^2 = 0.87$). There was also a positive linear relationship between total <16C FA, PA, and stearic acid with SFC (R^2 of 0.65, 0.67, and 0.42; $P < 0.001$). The backward elimination multivariate model started with PA, SA, OA, and <16C FA and only retained OA (all other $P > 0.15$). In conclusion, increasing oleic acid concentration decreased SFC at room temperature. The absence of and effect of PA in contrast to other data sets while modifying OA highlights the effect of the distribution of the data set on prediction of melting properties.

Key Words: unsaturated fat, buttergate

2202 Milk transfer efficiency of long- and very-long-chain unsaturated fatty acids from microencapsulated sources of fish, algae, and oleic acid oil. V. Sáinz de la Maza-Escola^{1,4,2}, A. Celemin Sarmiento^{3,1}, M. da Silva^{7,1}, A. Piva^{2,4}, E. Grilli^{2,5}, and J. E. Rico^{6,1}, ¹University of Maryland, College Park, MD, ²University of Bologna, Bologna, Italy, ³South Dakota State University, Brookings, SD, ⁴Vetagro Spa, Reggio Emilia, Italy, ⁵Vetagro Inc., Chicago, IL, ⁶University of Pennsylvania, Kennett Square, PA, ⁷Bonn-Rhein-Sieg University of Applied Sciences, Rheinbach, Germany.

Enrichment of milk fat with n-3 fatty acids is limited by ruminal hydro-genation, as well as by their preservation in bodily tissues via partitioning into membrane phospholipids. Our goal was to evaluate the effects of feeding microencapsulated forms of fish, algae, and oleic acid oils on milk transfer efficiency of unsaturated fatty acids. Nine Holstein cows (mean $\pm$ SD: 82 ± 18 DIM; 3.7 ± 0.9 lactations) were randomly assigned to treatments in a replicated 3×3 Latin square design with 9-d periods and 7-d washouts. At d -1, -2, 7, 8, and 9 of each period milk was sampled at each milking and composited for milk fatty acid analysis. Milk fatty acids were analyzed by GLC. Treatments were top-dressed daily and consisted of microencapsulated fish oil (FO; Prototype 6; 36% fish oil), algae oil (ALG; Prototype 39; 45% algae oil), and oleic acid (OA; Prototype 40; 20% oleic acid). The FO and ALG products delivered 25 g of the sum of n-3 (20:5 and 22:6), and OA provided 40 g of *cis*-9 18:1. Lipid supply was balanced at 226 g/cow per day. Data were analyzed under a general linear model with the random effect of cow (nested within treatment) and fixed effects of treatment, baseline, parity, and days in milk. The content of 20:5 in milk fat was highest in FO, relative to ALG and OA (0.049%, 0.026%, and 0.029%, respectively; $P < 0.01$), whereas 22:6 was higher in ALG, relative to FO and OA (0.048, 0.025, and 0.01 g/d, respectively; $P < 0.01$). No differences were detected for *cis*-9 18:1 content in milk. Milk transfer efficiency (TE) of 20:5 was 5.05% in cows receiving the FO treatment. For 22:6, TE reached 4.45% in the FO group and 3.13% in the ALG group. Milk TE of sum of n-3 FA was 4.83% and 5.96% for ALG and FO supplements, respectively. Cows in OA showed a milk TE of 63.5% for *cis*-9 18:1. Our results confirm the limited incorporation of absorbed n-3 FA into milk fat. Although overall incorporation remains low, the use of microencapsulated supplements may enhance FA bioavailability. The findings suggest that chain length and degree of saturation may affect the efficiency of n-3 FA transfer into milk.

Key Words: milk fat, n-3 fatty acids, oleic acid

2203 Effects of soybean meal enriched with soybean oil in Holstein cows. L. C. Ayala* and F. C. Cardoso, *University of Illinois, Urbana, IL.*

The combination of soybean meal with soybean oil may provide a cost-effective alternative to supply energy to dairy cattle. Twelve multiparous Holstein cows (BW = 643 ± 32 kg, DIM = 69.4 ± 14 d) were randomly assigned to 1 of 4 dietary treatments in a replicated ($n = 3$) 4×4 Latin square design balanced for carryover effects. Periods had 14 d of adaptation and 7 d of sampling. Treatments were a basal diet with canola (CAN), a basal diet with soybean meal (SBM), a basal diet with SBM and soybean oil (1.5% of dietary DM) as mash (MAS), and a basal diet with SBM and soybean oil (1.5% of dietary DM) as pellet (PEL). The TMR was fed to once daily. Cows were milked 2 times per day, milk weights were recorded at each milking, and milk samples from 20 and 21 d of each period were analyzed using mid-infrared procedures. Statistical analyses were conducted using the MIXED procedure of SAS. Cow was considered as a random effect. Treatment, square, and period were considered fixed effects. Three single-degree-of-freedom

contrasts were used: SBM vs. CAN, SBM vs. the average of MAS and PEL, and MAS vs. PEL. Cows in MAS and PEL had lower DMI (-2.1 and -1.8 kg/d, respectively; $P = 0.04$), and higher milk efficiency ($+0.22$ and $+0.14$, respectively; $P = 0.02$) compared with SBM (22.7 kg/d and 1.67, respectively). Cows in PEL and MAS had lower 3.5% FCM yield (-3.3 and -2.1 kg/d, respectively; $P = 0.02$), lower milk fat proportion (-0.52% and -0.34% percentual points, respectively; $P \leq 0.0001$), and lower milk in fat yield (-0.19 and -0.17 kg/d, respectively, $P = 0.003$) compared with SBM (39.3 kg/d, 3.87%, 1.42 kg/d, respectively). Cows in CAN had lower ECM yield (-3.9 kg/d; $P = 0.007$), lower 3.5% FCM yield (-2.7 kg/d; $P = 0.05$), lower milk fat yield (-0.15 kg/d; $P = 0.03$), and lower milk fat proportion (-0.25% percentual points; $P = 0.01$) compared with SBM (38.7 kg/d, 39.3 kg/d, 1.42 kg/d, 3.87%, respectively). Cows in PEL had lower milk yield (-1.7 kg/d; $P = 0.03$) and tended to have lower milk fat proportion (-0.18% percentual points; $P = 0.09$) compared with MAS (37.4 kg/d, 3.53%, respectively). In conclusion, DMI was reduced by the inclusion of soybean oil in MAS and PEL, and efficiency was higher. Cows in CAN, MAS and PEL had reduced milk components compared with SBM.

Key Words: soybean oil, pellet, performance

2204 Effects of n-3 fatty acid supplementation during the transition period on blood plasma inflammatory markers. H. L. Reisinger¹, M. L. Miller¹, L. K. Mamedova¹, G. A. Contreras², A. L. Lock¹, and B. J. Bradford¹, ¹Department of Animal Science, Michigan State University, East Lansing, MI, ²Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI.

Our objective was to evaluate the effect of supplementation of a calcium salt enriched in eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) on inflammation throughout the transition period. Multiparous Holstein cows ($n = 35$) were enrolled in a randomized incomplete block design. Treatments were a control diet containing a calcium salt of palm oil fed during the close-up (CU) and fresh (FR) periods at 0.60% diet DM (CON) or a diet containing a calcium salt enriched in EPA and DHA fed during the CU and FR periods at 0.90% diet DM (N3). Dietary supply of palmitic, oleic, and linoleic acids was balanced across treatments. CU period treatments were fed beginning 21 d before due date and FR period treatments were fed for the first 23 DIM. Blood was collected before supplementation, once before calving, and twice weekly until 23 DIM to assess circulating leukocytes (QScout), acute phase proteins (ELISA), and cytokines (bead-based ELISA). Data were analyzed using the random effect of block and cow nested within block, and fixed effects of treatment, time, parity, bovine leukemia viral status, and their interactions. Treatment tended to interact with time for circulating monocyte concentrations ($P = 0.08$) and immature neutrophil proportions ($P = 0.05$) although there were no treatment differences within specific time points. The N3 diet reduced lipopolysaccharide-binding protein (LBP) concentration ($P = 0.02$), whereas haptoglobin was not affected ($P = 0.96$). The N3 diet increased IL-1 α ($P = 0.04$) and VEGF-A ($P = 0.04$) concentrations and tended to reduce IL-4 concentration ($P = 0.07$). Treatment interacted with time for MCP-1 ($P = 0.03$) and TNF- α ($P < 0.01$), with N3 increasing concentrations at 3 DIM. Treatment also interacted with time for IL-10, however there were no treatment effects at any single time point. Treatment had no effect on IFN- γ , IL-6, IL-36RA, and MIP-1 α ($P > 0.10$). In conclusion, supplementation of EPA and DHA had subtle effects on systemic inflammation during the transition period, but did not dampen the inflammatory response after calving.

Key Words: immune response, docosahexaenoic acid, dietary fat

2205 Effects of diets designed to differ in the supply of de novo precursors and preformed fatty acids on milk fat yield during the summer. J. I. Norato* and A. L. Lock, *Michigan State University, East Lansing, MI.*

Seasonal variations in milk fat content are well documented, with lower levels typically observed during the summer months. However, limited research has focused on mitigating these seasonal declines. Milk fat comprises fatty acids (FA) from de novo and preformed sources. Our objective was to determine the effects of increasing the dietary supply of de novo precursors and preformed FA on milk production during the summer months. Twenty-six multiparous Holstein cows (131 ± 38 DIM) were used in a crossover design with 2 consecutive 35 d periods, during which weekly samples and data were collected. Treatment diets (DM basis) were: a control diet (CON, 30.6% starch, 3.80% sugar, and 4.14% total FA) and an increased milk fat precursor diet (MFP, 25.9% starch, 5.50% sugar, and 4.63% total FA), maintaining similar forage NDF (19.5% DM) and CP (16.5% DM). We targeted milk fat with the MPF diet using a multifaceted approach involving lower starch, higher sugar, increased amino acids and buffer, and different supplemental FA, which reduced dietary stearic and increased palmitic and oleic acids. The statistical model included the random effect of cow within square and the fixed effects of diet, period, square, and their interactions. Results are presented as CON vs. MFP. Compared with CON, MFP increased the yields of milk fat (1.56 vs. 1.69 kg/d, $P < 0.01$), 3.5% FCM (46.1 vs. 48.2 kg/d, $P < 0.01$), and ECM (45.8 vs. 47.4 kg/d, $P < 0.01$). However, MFP tended to decrease milk protein yield (1.40 vs. 1.38 kg/d, $P = 0.09$) and did not affect milk yield (48.1 vs. 48.2 kg/d, $P = 0.80$), lactose yield (2.31 vs. 2.33 kg/d, $P = 0.50$), or body weight (727 vs. 724 kg, $P = 0.32$). We also observed treatment-by-period interactions for the yields milk fat, 3.5% FCM, and ECM, with a greater response to MFP in period 1 than in period 2. In period 1, MFP increased the yields of milk fat, 3.5% FCM, and ECM by 0.20, 3.05, and 2.44 kg/d, whereas in period 2, these increases were 0.05, 1.22, and 0.79 kg/d, respectively. In summary, increasing the dietary supply of de novo precursors and preformed FA improved the yields of milk fat, 3.5% FCM, and ECM without affecting body weight.

Key Words: oleic acid, palmitic acid, milk fat

2206 The relationship between de novo fatty acids in bulk tank milk samples and milk components on Michigan dairy farms. J. M. dos Santos Neto*, K. Doneth, and A. L. Lock, *Michigan State University, East Lansing, MI.*

Milk fat is derived from 2 fatty acid (FA) sources: de novo FA (DNO), synthesized in the mammary gland, or preformed FA (PRE), obtained from the diet. Mixed FA (MIX) can be derived from either source. Our objective was to evaluate the relationship between bulk tank DNO and milk components on Michigan dairy farms. We used an extensive data set, including 436,058 daily bulk tank samples collected from 528 farms between 2022 and 2024. We had access to daily data for the following milk components: milk fat, protein, lactose, somatic cell count (SCC), DNO, MIX, and PRE. We calculated monthly farm averages per year, resulting in 18,819 observations for each component. Samples were subsequently divided into 2 categories based on bulk tank DNO content, creating 2 treatment groups: the upper 50% classified as high DNO (HDNO, $n = 9,409$) and the lower 50% as low DNO (LDNO, $n = 9,410$). Statistical analysis was performed using a mixed model, considering repeated measures, the random effect of farm, and the fixed effects of treatment, time, and their interactions. The Bonferroni correction was applied to all comparisons. Across the data set, samples averaged $4.0 \pm 0.32\%$ fat, $0.98 \pm 0.11\%$ DNO, $1.21 \pm 0.16\%$ MIX, and $1.52 \pm 0.17\%$

PRE. We observed interactions between treatment and time for all components ($P < 0.01$) except SCC ($P = 0.71$); these interactions did not alter treatment responses within months over years. Main effects are reported here. Compared with LDNO, HDNO increased fat (4.13% vs. 3.96%), protein (3.21% vs. 3.15%), DNO (1.02% vs. 0.95%), and MIX (1.25% vs. 1.17%, all $P < 0.01$), had no effect on lactose (4.69%, $P = 0.67$) or PRE (1.53%, $P = 0.44$), and decreased SCC (177 vs. 179K cells/mL, $P = 0.02$). In conclusion, associations were observed between increased DNO and increased milk components and reduced SCC in bulk tank milk samples, suggesting that monitoring DNO has potential as a valuable dairy management benchmark indicator. Future research aims to investigate HDNO, LDNO, and their relationships with farm management, diets, and environmental variables, expanding the opportunity for using DNO as a decision-making tool for dairy herd management.

Key Words: benchmark, de novo, milk fat

2207 Identifying inhibitors of a microbial enzyme responsible for milk fat depression in dairy cows. M. Saldivia* and T. Hackmann, *University of California, Davis, Davis, CA.*

Alteration in the biohydrogenation of fatty acids by rumen bacteria producing antilipogenic fatty acids has been proposed as responsible for milk fat depression in dairy cows. Recently, we proposed that we could alleviate milk fat depression by using inhibitors of enzymes forming these fatty acids. Here we test 4 potential inhibitors against *Cutibacterium acnes*, a rumen bacterium that produces the antilipogenic fatty acid *trans*-10,*cis*-12 CLA. For this purpose, we prepared Balch tubes with 9 mL of DSMZ 104 medium and 1 mL of linoleic acid, resulting in a final concentration of 0.5 g linoleic acid/L of medium. These tubes were inoculated with 0.1 mL of previously cultured *C. acnes* and incubated at 39°C. Cell extracts were harvested when the cultures reached an optical density of 1.7 at 600 nm, and were then exposed to different potential inhibitors based on those used for similar enzymes. Items CuSO_4 , FeSO_4 , polyethylene glycol 400, and phenelzine sulfate were tested at different concentrations using spectrophotometry at 233 nm. The reaction rate ($\text{mol} \cdot \text{L}^{-1} \cdot \text{min}^{-1}$) and enzyme activity were calculated. At a concentration of 2 mM, CuSO_4 inhibited production of *trans*-10,*cis*-12 CLA by 98% (4.4 SEM). For FeSO_4 , the inhibition was 90% (4.8 SEM), and for phenelzine sulfate, it was 87% (6.3 SEM). Polyethylene glycol was less effective; it inhibited activity only by 34% (2.9 SEM) at 2.4 M and had no effect at other concentrations. These findings suggest that inhibitors from different classes, such as divalent cations and hydrazine, can be used to inhibit enzyme activity in *C. acnes* cultures, showing potential for rerouting the biohydrogenation pathway and preventing the production of fatty acids linked with milk fat depression in dairy cows.

Key Words: milk fat depression, *trans*-10,*cis*-12 CLA, enzyme inhibitor

2208 Effects of raw and roasted high oleic soybeans on nutrient intake and digestibility of high-producing dairy cows. A. M. Bales* and A. L. Lock, *Michigan State University, East Lansing, MI.*

We determined the effect of feeding raw and roasted, ground high oleic acid soybeans (HOSB) on nutrient intake and digestibility of dairy cows. Thirty-six multiparous Holstein cows (45.6 ± 6.22 kg/d of milk; 110 ± 61 DIM) were randomly assigned to treatment sequences in a 4×2 truncated Latin square design with 35-d periods. Treatments were: control (CON), 16% roasted HOSB (RST), 16% raw HOSB (RAW-D), and 16% raw HOSB + by-pass protein (RAW-U). High oleic acid soybeans replaced conventional soybean meal and hulls in HOSB diets and by-pass protein replaced soybean meal in RAW-U to maintain diet

composition (% DM) of 21% forage NDF, 28% starch, and 17% CP. Total dietary fatty acid (FA) content was 2.8%, 4.9%, 5.1%, and 5.1% DM, respectively. The statistical model included the random effect cow within square and fixed effects of square, treatment, and period. Pre-planned contrasts were the overall effect of SOY {CON vs. HOSB [1/3 (RST + RAW-D + RAW-U)]}, effect of roasting {RST vs. RAW [1/2 (RAW-D + RAW-U)]}, and effect of protein (RAW-D vs. RAW-U). Order of results is: CON, RST, RAW-D, RAW-U. Feeding of SOY increased intake (kg/d) of DM (28.4, 29.0, 29.1, 29.1; $P = 0.04$) and NDF (9.18, 9.50, 9.56, 9.52; $P < 0.01$) and intake (g/d; all $P < 0.001$) of 16-carbon (70.8, 109, 106, 106), 18-carbon (378, 1169, 1199, 1169), and total FA (460, 1306, 1334, 1302). Feeding of SOY increased digestibility (%) of NDF (52.9, 56.0, 58.7, 59.7; $P < 0.001$) and decreased (all $P < 0.01$) digestibility of 16-carbon (72.6, 69.1, 66.8, 69.3), 18-carbon (78.4, 72.8, 67.2, 69.9), and total FA (76.3, 72.1, 66.8, 69.5). Feeding of SOY increased absorption (g/d; all $P < 0.01$) of 16-carbon (51.1, 74.8, 71.2, 74.9), 18-carbon (289, 852, 808, 819), and total FA (339, 950, 897, 920). Compared with RAW, RST decreased digestibility of DM (70.2, 70.7, 71.6, 72.0; $P = 0.02$) and NDF ($P < 0.01$), and increased digestibility and absorption of 18-carbon and total FA (all $P < 0.05$). Compared with RAW-D, RAW-U increased digestibility ($P = 0.02$) and absorption ($P = 0.01$) of 16-carbon FA. Overall, inclusion of 16% DM HOSB increased nutrient intake and FA absorption. Roasting HOSB increased digestion and absorption of dietary FA compared with raw HOSB.

Key Words: oleic acid, soybean, digestibility

2209 Milk fatty acid composition from Holstein cows fed fat supplements with increasing amounts of palmitic acid. J. K. Drackley¹*, A. M. Dickerson¹, L. Garcia¹, F. C. Cardoso¹, S. L. Burtnett², K. J. Harvatine², J. Albrecht³, O. R. Drehmel³, J. R. Loften³, and M. F. Scott³, ¹University of Illinois Urbana-Champaign, Urbana, IL, ²Penn State University, State College, PA, ³Actus Nutrition, Eden Prairie, MN.

Milk fatty acid (FA) profiles change due to addition of fat supplements, but the incremental effect of increasing palmitic acid (PA) has not been widely studied. Lactating Holstein cows ($n = 20$; 117 ± 21 DIM) were assigned to 1 of 5 treatments in a replicated 5×5 Latin square design balanced for carryover effects. Diets were formulated with no added fat (CON), or the basal diet supplemented with 2% (DM) fatty acid supplements containing 9% C18:1 and 35% C16:0 (PA35), 50% C16:0 (PA50), 65% C16:0 (PA65), or 80% C16:0 (PA80), with the remainder being mostly C18:0. Each treatment period lasted 21 d. Milk samples collected at each milking on d 20 and 21 of each period were composited by day. Milk fat was extracted from milk samples and methyl esters of FA were prepared by base-catalyzed transesterification. Fatty acid methyl esters were quantified by GLC. Data were analyzed using the MIXED procedure of SAS. Contrasts were CON vs. fat supplementation and the linear, quadratic, and cubic effects of increasing PA. Significance was $P < 0.05$. Increasing dietary PA increased content of C16:0 in milk fat compared with CON, and linearly increased PA (31.6, 31.2, 33.4, 35.9, and 38.8 g/100 g FA), while linearly decreasing C18:0 (9.1, 10.7, 10.0, 9.4, and 8.6 g/100 g FA) and *cis*-9 C18:1 (17.6, 18.8, 18.5, 17.6, and 16.6 g/100 g FA). Fat supplements decreased content of total de novo synthesized FA relative to CON, and PA linearly decreased de novo FA (28.8, 26.7, 26.2, 25.4, and 24.7 g/100 g FA). Milk FA yields (g/d) generally followed the same patterns, except that many responses were quadratic because milk fat yield was maximized by PA65. Yield of C16:0 (400, 423, 469, 532, and 547 g/d) was greater for fat supplements and increased quadratically ($P = 0.08$) with increasing PA. Yield of C18:0 (114, 147, 143, 138, and 119 g/d) was greater with FA supplementation

and decreased quadratically with increasing PA. Yields of de novo FA (346, 337, 348, 352, 332 g/d) were not affected by FA but increased quadratically with increasing PA. The PA inhibited content of de novo FA more than C18:0 and decreased most other FA in milk fat.

Key Words: milk fat, dietary fat, palmitic acid

2211 Effects of high oleic acid soybean expeller meal on milk production of dairy cows. J. E. Parales-Girón*, L. C. Worden, J. I. Norato, J. M. dos Santos Neto, A. M. Bales, and A. L. Lock, Michigan State University, East Lansing, MI.

We recently reported that increasing dietary inclusion of high oleic soybeans positively affects milk production of high-producing dairy cows. However, the effect of increasing dose of high oleic soybean expeller meal (HOEM) on feed intake and the yields of milk and milk components has yet to be determined. Twenty-four multiparous Holstein cows (50.6 ± 5.10 kg/d of milk; 107 ± 25 DIM) were randomly assigned to treatment sequences in a replicated 4×4 Latin square design with 21-d periods. Treatments were increasing doses of HOEM at 0%, 6%, 12%, and 18% diet DM. High oleic soybean expeller meal (5.63% DM total fatty acids [FA]; 79% oleic acid) replaced heat-treated soybean meal and soyhulls to maintain diet nutrient composition (% DM) of 29% NDF, 20% forage NDF, 28% starch, and 17% CP. Total dietary FA content was 3.42%, 3.70%, 3.98%, and 4.26% DM, respectively. The statistical model included the random effects of cow within square and the fixed effects of treatment, period, square, and their interactions. Pre-planned contrasts included the linear (L), quadratic (Q), and cubic (C) effects of increasing HOEM. Order of results is: 0%, 6%, 12%, and 18% HOEM. Increasing HOEM affected the yields (kg/d) of milk (47.2, 47.8, 48.4, 48.9; $L P < 0.01$), 3.5% FCM (51.5, 51.3, 52.3, 52.6; $L P < 0.01$), ECM (51.3, 51.0, 52.0, 52.1; $L P = 0.02$), milk fat (1.92, 1.89, 1.94, 1.94; $L P = 0.07$, $C P = 0.04$), and MUN content (12.9, 12.1, 12.2, 11.5 mg/dL; $L P < 0.01$, $C P = 0.01$). However, treatment did not affect DMI or milk protein yield (both $P \geq 0.25$). Increasing HOEM decreased the yields (g/d) of de novo (467, 449, 452, 437; $L P < 0.01$, $C P = 0.02$) and mixed milk FA (789, 746, 747, 713; L and $C P < 0.01$) but increased preformed milk FA (544, 575, 627, 662; $L P < 0.01$, $C P = 0.03$). In summary, increasing dietary inclusion of HOEM up to 18% diet DM linearly increased yields of milk, 3.5% FCM, and ECM, tended to increase milk fat, but did not alter milk protein. Increasing HOEM affected milk FA sources, suggesting that improvements in milk fat responses to HOEM are feasible. Further research is needed to explore nutritional strategies to increase milk fat without reducing de novo and mixed FA when feeding HOEM.

Key Words: oleic acid, soybean expeller meal, milk fat

2212 Effects of palmitic acid and high oleic acid soybeans on milk production of high-producing dairy cows. A. M. Bales* and A. L. Lock, Michigan State University, East Lansing, MI.

Our objective was to determine how the supply of palmitic acid (PA) and high oleic acid soybeans (HOSB) altered the yields of milk fat and milk FA sources of high-producing dairy cows. Twenty multiparous Holstein cows (45.9 ± 4.7 kg/d of milk; 136 ± 18 DIM) were randomly assigned to treatment sequences in a replicated 4×4 Latin square design with 14-d periods. Treatments were 1) control (Trt1), 2) 1.5% DM PA (Trt2), 3) 16% DM HOSB (Trt3), and 4) 1.5% DM PA + 16% DM HOSB (Trt4). Palmitic acid replaced soybean hulls and HOSB (ground and roasted) replaced conventional soybean meal and hulls to maintain diet composition (%DM) of 21% forage NDF, 25% starch, and 17% CP.

Total FA content of treatments was 1.56%, 2.92%, 4.03%, and 5.42% DM, respectively. The statistical model included the random effect of cow within square, the fixed effects of period, PA, and HOSB, and the interaction of PA $\times$ HOSB. Order of results is: Trt1, Trt2, Trt3, and Trt4. There were no interactions observed for DMI or milk production responses. PA increased the yields (kg/d; all $P < 0.001$) of 3.5% FCM (44.3, 46.3, 49.3, 50.7), ECM (44.6, 46.2, 48.2, 50.1), and milk fat (1.63, 1.72, 1.80, 1.94), and tended to increase milk yield (41.2, 42.3, 44.2, 44.5 kg/d; $P = 0.07$) but did not affect DMI (31.6, 31.5, 32.1, 32.3 kg/d; $P = 0.99$) or milk protein yield (1.41, 1.44, 1.46, 1.46 kg/d; $P = 0.21$). High oleic acid soybeans increased DMI ($P = 0.02$) and yields of milk, 3.5% FCM, ECM, milk fat and milk protein (all $P < 0.01$). For milk FA sources, there was an interaction for the yield of mixed FA (623^c, 710^a, 524^d, 661^b g/d [values with different superscripts differ]; $P = 0.01$). Palmitic acid did not affect yields (g/d) of de novo (439, 424, 424, 421; $P = 0.19$) or preformed FA (467, 477, 746, 739; $P = 0.77$). High oleic acid soybeans increased yields of preformed FA ($P < 0.001$) but not de novo FA ($P = 0.20$). In summary, both PA and HOSB increased yields of milk and milk fat. When combined we observed an additive effect (Trt4) as demonstrated with milk fat yield, which increased by 90, 170, and 310 g/d for Trt2, Trt3, and Trt4, respectively, compared with Trt1. This emphasizes the importance of supplying multiple FA sources to the mammary gland for increased milk fat synthesis.

Key Words: oleic acid, palmitic acid, milk fat

2239 Effects of balancing dietary 18-carbon fatty acids from high oleic soybeans or a commercially available stearic-acid-enriched supplement on milk production of mid-lactation dairy cows. B. A. Seelenbinder*, J. E. Parales-Girón, A. M. Bales, and A. L. Lock, *Michigan State University, East Lansing, MI.*

We determined the short-term effects of balancing dietary 18-carbon fatty acids (FA) from high oleic soybeans (15.4% DM total FA; 85%

oleic acid [C18:1]) or a commercially available stearic acid-enriched fat supplement (97.3% DM total FA; 52% stearic acid [C18:0]) on milk production of dairy cows. Thirty-two Holstein cows (49.7 ± 5.76 kg/d, 120 ± 33 DIM) were used in a crossover design experiment with 2 consecutive 14-d periods, with sampling the last 4 d of each period. Treatments were a diet containing 8% diet DM roasted and ground high oleic soybeans (HOS) or a diet including 2% diet DM of a stearic-acid-enriched fat supplement (SAT). Conventional soybean meal, heat-treated soybean meal and soyhulls were used in differing amounts to maintain diet nutrient composition of (%DM) 21% forage NDF, 28% starch, and 17% CP (46% RUP). A palmitic acid (C16:0)-enriched supplement was included in HOS to ensure similar dietary C16:0 content. Diets contained (% total FA) 24% vs. 23% C16:0, 3% vs. 24% C18:0, 37% vs. 13% C18:1, and 73% vs. 70% total 18-carbon FA for HOS and SAT, respectively. The statistical model included the random effect of cow within square and the fixed effects of treatment, period, square, and their interactions. Compared with SAT, HOS decreased DMI (31.2 vs. 32.0 kg/d; $P = 0.03$), increased the yields (kg/d) of milk (50.8 vs. 48.5; $P = 0.01$), milk protein (1.58 vs. 1.51; $P = 0.02$), milk lactose (2.49 vs. 2.39; $P < 0.01$), 3.5% FCM (55.1 vs. 53.0; $P = 0.04$), and ECM (54.4 vs. 52.1; $P = 0.03$), and tended to increase milk fat (2.05 vs. 1.97; $P = 0.07$). Additionally, HOS increased short-term BW gain (0.78 vs. -0.01 kg/d; $P < 0.01$) compared with SAT. Despite balancing diets for total, 16-, and 18-carbon FA in HOS and SAT, supplying 18-carbon FA mainly as oleic acid from high oleic soybeans improved milk and milk component yields compared with supplying stearic acid from a commercially available stearic acid-enriched supplement. This emphasizes the importance of considering individual 18-carbon FA in diet formulations rather than grouping them collectively.

Key Words: oleic acid, stearic acid, milk production

Small Ruminant 1: Posters

2213 Factors affecting milk yield, composition, and dairy processing efficiency of 2 Italian dairy sheep breeds: Preliminary results. S. Graziani¹, M. C. Fabbri¹, F. Sirtori¹, J. C. C. Panetto^{*1,4}, R. Bozzi¹, G. Stocco², A. Zanotti², D. Valoppi², C. Cipolat-Gotet², I. Tedeschi², S. Biffani², B. Castiglioni³, and F. Tiezzi¹, ¹*Università degli Studi di Firenze, Firenze, Italy*, ²*Department of Veterinary Science, Università degli studi di Parma, Parma, Italy*, ³*Institute of Agricultural Biology and Biotechnology, National Research Council, Milano, Italy*, ⁴*Embrapa Dairy Cattle, Juiz de Fora, Brazil*.

High production costs and seasonality of production are challenging the dairy sheep industry, requiring the identification of critical points and biological mechanisms to improve resilience, sustainability, and feed efficiency. The aim of this study was to evaluate factors affecting the variability of production and quality traits on a total of 144 animals belonging to 2 Italian sheep breeds: Comisana (n = 62) and Massese (n = 82). Phenotypic data were collected on a commercial farm in central Italy across different seasons. Recorded traits included productivity (kg of milk/day), milk composition (fat, protein, lactose, total solids), and cheese recovery efficiency (casein content, casein index, somatic cell counts and pH), with a focus on cheese yield and the recovery of milk components in the curd. Records (n = 432) were validated based on information provided by Associazione Nazionale della Pastorizia (Asso. Na.Pa.), and analyses of variance were applied to assess the impact of different effects on the analyzed traits. Season, breed, parity, stage of lactation and interaction between breed and season were considered as fixed effects, while the animal effect was considered as random. The effect of breed was significant for casein index ($P < 0.05$), pH ($P < 0.001$) and milk urea nitrogen ($P < 0.01$). On average, milk from Massese ewes had a casein index of 75.5, urea nitrogen level of 45.9 mg/dL, and a pH of 6.50. Conversely, milk from Comisana ewes had a casein index of 75.3, urea nitrogen level of 48.8 mg/dL, and a pH of 6.58. Season and interaction between breed and season have affected most traits (fat, protein, lactose, casein, total solids, dry matter cheese yield, recovery of total solids in the curd, milk yield, pH, and urea), along with stage of lactation. The findings from this preliminary analysis suggest that differences among sheep breeds may be exploited in the search for better adaptable herds in specific environmental conditions. Further studies are still ongoing as part of the project.

Key Words: animal resilience, cheese recovery efficiency, Comisana, environmental effect, ewe, Massese

2214 Neonatal care challenges and management practices in US meat and dairy sheep operations: Insights from a national survey. E. Josse^{2,1}, K. Froehlich³, and M. Rovai^{*1}, ¹*South Dakota State University, Brookings, SD*, ²*L'Institut Agro Rennes-Angers, Rennes, France*, ³*American Sheep Industry Association's Young and Emerging Entrepreneurs group, Englewood, CO*.

Neonatal care is crucial for meat and dairy sheep industries, as lamb survival directly affects farm profitability and sustainability. Neonatal mortality rates range from 15% to 25% worldwide, posing challenges for farmers. Proper colostrum management, disease prevention, and optimal lambing conditions are essential to reducing early-life losses. A nationwide survey of US sheep farmers (607 respondents) collected data on flock size, management practices, and lamb mortality causes. The survey consisted of 4 sections with 24 questions, primarily nominal

and ordinal, with a few open-ended questions. It was distributed via social media (Facebook sheep groups), in-person breeder meetings, and newsletters from the Wisconsin Sheep Extension, South Dakota Sheep Extension, and American Sheep Industry, targeting 100 Facebook groups across Canada, the United States, and Mexico in English, French, and Spanish. Responses were analyzed in R Studio (version 4.3.2) using descriptive and exploratory analysis. Results showed that most respondents were in the North-Central and South-Central regions, with 57% focused on meat production, while a growing number engaged in dairy production. Small flocks (<100 head) were the most common but accounted for a minor fraction of the total US sheep population, highlighting the importance of larger operations. The survey identified gaps in birth management, particularly in lamb navel disinfection, which was not consistently performed (n = 206/575, 36%), especially among large flock operators. The leading causes of lamb mortality were disease (14%), harsh weather (13%), accidents (10%), and poor maternal aptitude (8.2%), emphasizing the need for better lambing protocols and farmer education. Colostrum management was a key focus, revealing that 45% of farmers supplemented colostrum powder, whereas 35% used stored ewe colostrum. However, only 2% (n = 14/575) tested colostrum quality, indicating a gap in awareness and accessibility to quality assessments. More educational material and improved access to colostrum testing could significantly enhance lamb survival. Future research should explore best practices for reducing mortality and increasing producer adoption of evidence-based husbandry techniques.

Key Words: neonatal lamb survival, sheep management practice, colostrum quality assessment

2215 Metabolizable protein requirements for maintenance and growth in female Saanen goats in the late growth stage. P. A. Sampaio^{*1}, Q. A. Alves², G. M. Moreira², B. Biagioli², K. T. Resende², and I. A. M. A. Teixeira¹, ¹*University of Idaho, Twin Falls, ID*, ²*UNESP—Universidade Estadual Paulista, Jaboticabal, Sao Paulo, Brazil*.

Estimating protein requirements is essential for improving nitrogen utilization in dairy goat diets, thereby enhancing economic efficiency and environmental stewardship. The objective of this study was to determine the MP requirements for maintenance and growth in female Saanen goats weighing from 30 to 45 kg. Data from 2 comparative slaughter experiments conducted at the Unesp, Jaboticabal, campus were used. In the first experiment, 24 goats with an initial BW of 30 kg were used to estimate MP requirements for maintenance (MPm). Goats were subjected to 3 intake levels (ad libitum, 30% restriction, and 60% restriction). Six goats were slaughtered at the beginning of the experiment to estimate the initial body composition. The remaining 18 goats were randomly assigned to 6 blocks of 3 goats each, with treatments. The MPm was estimated from the regression of retained body protein on MP intake (MPI). The MPm was estimated at 2.1 g/kg^{0.75} BW. In the second experiment, 18 goats with an initial BW of 30 kg were used to estimate MP for growth (MPg). Goats were fed ad libitum and slaughtered at a target BW of 30 kg, 38 kg, and 45 kg. The net protein requirement for growth (NPg) was derived from an allometric relationship between body protein and empty body weight (EBW). The MPg was calculated as NPg divided by the efficiency of MP use (Kpg), resulting in the equation: $MPg = 314.585 \times EBW^{(-0.1677)}$ (g/kg of EBW gain). Our findings contribute to refining protein requirements for Saanen goats, thereby

improving protein nutrition strategies in dairy goat production systems by enhancing nitrogen utilization efficiency.

Key Words: protein nutrition, body composition, nitrogen

2216 Oxidative status of ewe colostrum related to mother age, body condition, and feeding season. F. Belkasmi*^{1,2}, F. Baali³, C. Mouffok⁴, and L. Semara², ¹Langston University, Langston, OK, ²Ghardaïa University, Ghardaïa, Algeria, ³Bordj Bou Arreridj University, El Anasser 34030, Bordj Bou Arreridj, Algeria, ⁴Ferhat Abbas University, Setif, Algeria.

The defense mechanisms in ewes' colostrum rely not only on passive immunity but also on its antioxidant properties, preventing oxidative cell damage caused by free radicals. The present study was conducted to evaluate antioxidant potential in ewes' colostrum to analyze antioxidant variability according to season, age and BCS of ewes. Colostrum samples from 134 healthy Ouled Djellal ewes were collected within 24 h postpartum at 3, 6, 9, 12, and 24 h. After centrifugation, the lipid layer was removed using a pipette, and the aqueous fraction was analyzed for antioxidant activity. The analyses included total antioxidant capacity (TAC), α, α -diphenyl- β -picrylhydrazyl (DPPH) radical scavenging activity, and catalase (CAT) activity. The descriptive analysis of the collected data reveals that the data does not follow a normal distribution. Consequently, a non-parametric test, specifically the Kruskal-Wallis test, was used to assess the influence of various factors, such as lambing

season, ewe age, and body condition at lambing. This test allows for the comparison of group distributions without assuming data normality. The statistical significance threshold was set at $P < 0.05$. This approach ensures a robust analysis of the effects of the studied factors, despite the non-normality of the data. The results revealed significant differences in antioxidant activity dynamic across the milking periods during the first 24 h ($P < 0.001$, $P < 0.01$, and $P < 0.05$ for TAC, DPPH, and CAT respectively). DPPH levels were highest immediately after lambing and gradually declined overtime, whereas CAT and TAC showed the opposite trend, with their activity increasing with time. Our results demonstrate that season, age and BCS had significant effects on antioxidant activities. Unlike TAC and DPPH, CAT was highly influenced by season ($P < 0.000$) which may be linked to the sensitivity of enzymatic activities to temperatures. The highest antioxidant activity according to age was seen at yearling for DPPH (50.34 ± 4.08) and in young ewes for TAC ($1,045.90 \pm 148.18$) and CAT (3.77 ± 0.52). However, BCS affects different antioxidant activities. Highest values were in fat ewes for TAC ($1,110.32 \pm 156.33$), in moderate ewes for DPPH (52.87 ± 2.73) and in lean ewes for CAT (3.67 ± 0.37). Our study fills a gap in knowledge by providing estimation of antioxidant activities in Ouled Djellal ewes' colostrum and its variability dynamic according to animals and season; it can also be used to improve productivity of sheep and overall health management. Nevertheless, further research is needed to deepen our knowledge and optimize practical applications.

Key Words: antioxidant activity, colostrum, ewe

ADSA-USD Competition: Original Research Poster Presentations

2217 Evaluation of casein micellar dissociation and functionality after applying novel processing method. G. E. Lewis, A. Fuchs*, and D. Stroinski, *University of Wisconsin–River Falls, River Falls, WI.*

In milk, casein proteins orientate themselves into spherical micellar structures with typical diameters ranging from 80 to 400 nm. Previous research has shown that milk with the addition of emulsifying salt (ES) coupled with high-pressure homogenization (HPH) induces an unprecedented amount of casein micellar dissociation. This research aims to quantify the extent of casein micellar dissociation in milk following the treatment of ES coupled with HPH (ES+HPH) and to evaluate the functionality of these casein proteins following the treatment. To quantify the extent of micellar dissociation, skim milk solutions (20% vol/vol) were prepared with a varying amount of treatment (no processing, only ES 100 mM sodium hexametaphosphate; only HPH [at 300 MPa]; and ES+HPH), put through varying filter sizes (0.22 μ m, 0.05 μ m), and analyzed using SDS-PAGE. In the control group (20% skim milk), $9.35\% \pm 2.53\%$ casein protein remained post-filtration through a 0.05 μ m filter. Alternatively, $93.2\% \pm 7.71\%$ casein proteins were present in ES+HPH samples post-filtration through a 0.05 μ m filter. These results show that applying ES coupled with HPH to milk causes a significant reduction in the size of casein micelles. One benefit of the dissociation of casein micelles is that it exposes more of the hydrophobic proteins from the cores of the micelles, which, in theory, would form more stable foams. Using this knowledge, the foam expansion index (FEI) of skim milk was also monitored for the various treatments (no processing, only ES, only HPH, and ES+HPH) at set intervals after foaming (0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, and 8 h). The control group FEI was $33.33\% \pm 14.43\%$ at 0 h and $21.67\% \pm 2.887\%$ after 8 h. In comparison, skim milk treated with ES coupled with HPH had FEI values of $138.3\% \pm 12.58\%$ at 0 h and $113.3\% \pm 5.774\%$ after 8 h. This data demonstrates that ES coupled with HPH leads to a significant increase in the volume and stability of foam when compared with the control group. Future steps in this research would be to evaluate different applications related to these processing methods such as digestibility.

Key Words: casein micelles, emulsifying salt, high-pressure homogenization

2218 The effects of black soldier fly larvae meal on in vitro rumen fermentation characteristics and bacterial abundance. A. Ellis*, G. G. Begalli¹, A. F. Lima¹, M. Zarei², T. Fernandes¹, and J. S. Osorio¹, ¹*School of Animal Sciences, Virginia Tech, Blacksburg, VA*, ²*Virginia Seafood Agriculture Research and Extension Center, Virginia Tech, Hampton, VA.*

Protein is one of the most expensive nutrients in dairy cows' rations; therefore, there has been an increased interest in alternative protein sources such as insect meal (IM). This study aimed to determine the influence of incremental levels of IM on in vitro rumen fermentation profile and bacterial abundance. Rumen fluid was collected from 2 mid-lactation, multiparous cannulated dairy cows (~142 DIM). Then, filtered rumen fluid was diluted in Goering and Van Soest buffer under CO₂ flux. Buffered rumen fluid (20 mL) was added to test tubes and assigned to one of the treatments (DM basis): T1, 100% TMR (containing 200 mg of TMR); T2, 97.5% TMR + 2.5% IM; T3, 95% TMR + 5% IM; T4, 92.5% TMR + 7.5% IM; T5, 90% TMR + 10% IM. The test tubes were closed and placed in an incubator (39°C) with a shaking system set at 80 rpm for 0, 3, 6, 12, 24, and 48 h. Incubation was

terminated by placing test tubes on ice. The pH of all samples was measured immediately following termination and then frozen (–80°C) for later analysis of VFA, in vitro DM digestibility (IVDMD), bacterial abundance, and NH₃. Bacterial abundance was determined via RT-qPCR methods. A sample of IM was analyzed for CP (38.3%) and fat (39.1%). Data were analyzed using the MIXED procedure of SAS with preplanned orthogonal contrast to test linear and quadratic effects over the incremental levels of IM. A linear increase in NH₃ ($P = 0.05$) and valerate ($P = 0.02$) was observed across IM levels. In contrast, acetate ($P = 0.01$) and IVDMD ($P < 0.01$) linearly decreased over IM levels. A trend ($P = 0.09$) for a linear decrease in propionate was observed. There was a linear decrease ($P = 0.02$) in the abundance of *Butyrivibrio proteoclasticus* as the IM levels increased. Similarly, a trend ($P = 0.07$) for a linear decrease in *Anaerovibrio lipolytica* was observed. Ammonia and valerate increases suggest an increased rate of protein degradation. High fat content in IM may have contributed to the decrease in IVDMD, VFA, and some bacteria. These results suggest that IM with lower fat content may be more suitable for supplying protein while maintaining adequate rumen fermentation.

Key Words: insect meal, microbial abundance, VFA

2219 Utilization of whey protein phospholipid concentrate in model drinking yogurt. J. Pizarro*, J. Huang, and B. Vardhanabhuti, *University of Missouri Columbia, Columbia, MO.*

Whey protein ingredients are widely used due to their nutritional and functional properties. Whey protein phospholipid concentrate (WPPC), a coproduct from whey protein manufacturing, has not been fully utilized. Rich in phospholipids, WPPC may enhance creaminess and viscosity, properties particularly desirable in acidified dairy beverages. This study investigated the effects of acidification and WPPC sources on the physical properties of heated WPPC in model drinking yogurt system. The WPPC samples were obtained from 4 manufacturers (M1–M4). The WPPC dispersions (8% wt/wt protein, pH 7) were heated at 90°C for 40 min. Acidification was achieved through glucono-delta-lactone (GDL) addition at 0.15%, 0.25%, and 0.35%. The resulting systems were characterized for pH, particle sizes, and rheological properties. The zeta potential of the heated samples was also measured. Data were analyzed using ANOVA with means separation. All heated WPPC samples showed similar surface charge property with zeta potential values ranging from –23.7 to –24.9 mV ($P > 0.05$). Both WPPC source and GDL level significantly influenced particle sizes and rheological properties ($P < 0.05$). At 0.15% GDL, where the pH was nearest to the pI (isoelectric point), M4 exhibited the largest D_{4,3}, (37.3 mm) whereas M1 and M2 displayed the smallest sizes (9.25 and 13.1 mm, respectively). At 0.25% and 0.35% GDL, D_{4,3} ranged from 16.0 to 22.4 mm. All samples exhibited high shear thinning behavior with flow behavior indices ranging from 0.27 to 0.48 ($P > 0.05$). The M1 and M2 samples exhibited lower apparent viscosity with consistency indices (K) ranging from 1.39 to 2.90 Pa·sⁿ. Significantly larger K values were observed for M3 and M4 at 0.15 and 0.25% GDL with M4 reaching 17.4 Pa·sⁿ. These findings demonstrate that both WPPC source and the final pH significantly influenced aggregation formation and rheological properties. Proximity to the isoelectric point enhanced particle aggregation for specific sources, resulting in increased viscosity, suggesting strategic consideration of acidification parameters in beverage formulations.

2220 Effect of pulsed electromagnetic field therapy on milk quality in organic dairy cows. K. A. Young*, F. C. Pharo, and C. R. Seely, *University of New Hampshire, Durham, NH.*

Pulsed electromagnetic field therapy (PEMF), a noninvasive form of electromagnetic stimulation, is used in companion animal medicine to manage pain and promote healing. In organic dairy systems, where antibiotics and pharmaceuticals are limited, opportunity exists to explore alternative treatments to promote cow health. Our objective was to explore the effect of PEMF on milk yield and composition in organic dairy cows. Mid-lactation Jersey cows ($n = 36$) of various parities were randomly assigned to 1 of 3 treatments: (1) control (CON; $n = 12$); no PEMF, (2) once daily PEMF (PEMF1; $n = 12$), or (3) twice daily PEMF (PEMF2; $n = 12$). Cows were enrolled on the study for 10 consecutive days: 2 d of baseline (BL) where no treatments occurred, 3 d of treatment (TX), and 5 d of follow-up (FU). During TX, PEMF was given directly after AM (PEMF1 and PEMF2) and PM (PEMF2) milkings for 12 min; 8 min on the cow's topline, 2 min on the udder's right quarters, and 2 min on the left quarters. For the duration of the study, daily milk yield was recorded, and composite milk samples were collected at each milking for component analysis. Linear mixed models were created to assess the differences overtime between treatment groups for milk yield and components during BL, TX, and FU. Milk yield was similar between groups for BL and TX periods and did not change over time ($P > 0.3$). During FU, milk yield was greater ($P = 0.01$) in multiparous PEMF1 and PEMF2 cows (24.8 [23.7, 26.0] kg/d and 25.2 [23.8, 26.6] kg/d, respectively) compared with primiparous PEMF1 and PEMF2 cows (22.4 [21.0, 23.8] kg/d and 22.7 [21.5, 24.0] kg/d, respectively). There was no effect of treatment or time on milk components ($P > 0.1$). Additionally, SCC was similar between treatment groups for all periods ($P > 0.6$; CON = $1.13 [0.80, 1.94] \times 10^5$ cells/mL, PEMF1 = $1.17 [0.88, 2.05] \times 10^5$ cells/mL, PEMF2 = $0.78 [0.41, 1.19] \times 10^5$ cells/mL). Our findings suggest that PEMF may offer an opportunity to improve milk quality when antibiotics or pharmaceutical remedies are unavailable. Future investigations into the utility of PEMF in clinically ill dairy cattle are warranted.

Key Words: pulsed electromagnetic field therapy, milk quality, organic dairy

2221 Enhancing alfalfa cultivation with plasma-activated water: Impact on germination and growth. G. F. Helle, T. Brink, D. Manoharan, and Z. Wan*, *University of Wisconsin-Platteville, Platteville, WI.*

Plasma-activated water (PAW) is a promising agricultural innovation with potential benefits for dairy farming, particularly in forage production and herd health. This study examines its effects on alfalfa germination and growth, a key forage crop for dairy cattle. Cold plasma, a nonthermal technology, has applications in food safety, animal health, and agriculture. When applied to water, it creates PAW, which may enhance nutrient uptake, improve silage fermentation, and reduce harmful pathogens in water and feed. Plasma-activated water is produced by exposing water to high-voltage atmospheric cold plasma (HVACP) with a single donut electrode, generating reactive oxygen and nitrogen species (RONS) that enhance germination, plant development, and forage quality. Before irrigation with PAW, seeds underwent direct plasma treatment at 40 and 50 kV for up to 90 s. Germination was then tested using PAW produced at 50 kV with plasma exposure times up to 3 min. Direct plasma exposure to seeds can cause electroporation and seed coat erosion, potentially aiding water absorption and growth, whereas no significant increase in germination from direct plasma treatment was observed ($P > 0.05$). In contrast, PAW irrigation significantly improved

germination, likely due to a synergistic effect. This enhancement may result from oxidative stress-induced signaling pathways activated by RONS, stimulating early seedling development. Germination rates of alfalfa seeds treated at 50 kV for 90 s using a single donut treatment increased by 29.4% compared with the control. Plants watered with PAW showed greater biomass accumulation and elongation, likely due to improved metabolic activity and nutrient availability. The presence of RONS may act as a mild stressor, triggering adaptive responses that enhance plant resilience. PAW could serve as an eco-friendly alternative to chemical fertilizers, improving germination, nutrient uptake, and growth. For dairy farming, this may lead to higher forage yields and better feed quality, supporting herd nutrition and productivity. Future research should refine PAW application and assess its long-term effects on soil microbes and forage quality for sustainable dairy operations.

Key Words: plasma-activated water (PAW), high-voltage atmospheric cold plasma (HVACP), reactive oxygen and nitrogen species (RONS)

2222 Milk-derived peptide valine-proline-proline supplementation altered hepatic glucose, insulin, and lipid metabolism. E. Kolodziej*, J. R. Daddam, G. Ahmad, M. Sura, and Z. Zhou, *Department of Animal Sciences, Michigan State University, East Lansing, MI.*

Milk-derived bioactive peptides have been thoroughly researched for their metabolic regulatory effects in humans and rodents, but their impact on cows remains largely unexplored to this day. During early lactation, a large influx of fatty acids are mobilized from adipose tissue. Because of this, hepatocytes in the bovine liver are overwhelmed and experience metabolic stress. The objective of this study was to assess the impact of valine-proline-proline (VPP), a milk-derived peptide, on primary bovine hepatocytes exposed to fatty acids. control (CON): customized medium with amino acids, volatile fatty acids, fatty acids (FA), glucose, choline, insulin, and albumin concentrations equal to circulating levels in cows 4 d postpartum; or VPP: CON + 100 μ M VPP. After 72 h of culture, hepatocytes were harvested and RNA was extracted for RNA sequencing. Raw reads were trimmed (Trimmometry), aligned to the bovine reference genome (STAR), and assembled and quantified (Salmon). The differential gene expression (DEG) analysis was performed with DESeq2 (R environment). Genes with adjusted P -value (FDR) < 0.05 were considered differentially expressed genes (DEGs). Functional enrichment analysis of the DEGs were carried out using the ingenuity pathway analysis (IPA) with Z-score 2.0 as cutoff for significant pathways. A total of 18,107 transcripts corresponding to 5,975 genes were identified, among which 536 were unique DEGs (FDR < 0.05). Enrichment analysis revealed that Glucose Metabolism, Insulin Receptor Signaling, Peroxisomal Lipid Metabolism, and Fatty Acid β -oxidation are top-enriched pathways in VPP supplemented hepatocytes compared with CON. These results indicate that VPP supplementation altered hepatic glucose, insulin, and lipid metabolism. Quantification of genes and proteins within these pathways is necessary to determine the specific impact of these metabolic changes.

Key Words: VPP, transcriptomics, metabolism

2223 Aromatic amino acids supplementation improved immune response upon pathogen challenge in fresh cow granulocytes and monocytes. M. E. Gojcaj*, J. R. Daddam, G. Ahmad, M. Sura, and Z. Zhou, *Department of Animal Sciences, Michigan State University, East Lansing, MI.*

Aromatic amino acid (AAA) depletion results in the production of dysfunctional proteins, compromising immune cell function. Insufficient

intake during the early postpartum period significantly reduces AAA availability to circulating immune cells. However, its impact on granulocyte and monocyte function remains unknown. The objective of the study was to determine the extent to which rumen-protected aromatic amino acids (RP-AAA) alter phagocytosis and oxidative burst capacity of blood neutrophils and monocytes upon pathogen challenge. Twenty multiparous Holstein cows were enrolled right after parturition in a 21-d complete randomized study. Treatments were: control (CON, without RP-AAA, $n = 10$); or rumen-protected AAA (RP-AAA, 61 g tryptophan, 117 g phenylalanine, and 102 g tyrosine top-dressed daily, $n = 10$). Granulocyte and monocyte phagocytosis and oxidative burst were assessed in whole blood on 10 and 20 d postpartum. The phagocytic capacity and oxidative burst activity of peripheral granulocytes and monocytes were determined upon challenge with pHrodo Red *E. coli* BioParticles using an Attune CytPix flow cytometer (Thermo Fisher Scientific, Waltham, MA). Treatment effects were determined using PROC GLIMMIX in SAS. Compared with CON, monocyte phagocytosis ($P = 0.01$, 48.3% vs. 36.5%, SEM = 2.87) and oxidative burst ($P = 0.01$, 36.4% vs. 20.6%, SEM = 3.67) were both greater in RP-AAA supplemented cows. Similarly, a tendency for greater granulocyte phagocytosis ($P = 0.10$, 29.2% vs. 20.3%, SEM = 3.6) and a greater granulocyte oxidative burst ($P = 0.02$, 48.7% vs. 31.1%, SEM = 4.86) was also observed in RP-AAA cows compared with CON. However, the mean fluorescent intensity of oxidative burst was not different between RP-AAA and CON cows for granulocytes and neutrophils. These results suggest that RP-AAA supplementation during early lactation can be beneficial for granulocyte and monocyte to mount better bactericidal response upon challenge.

Key Words: aromatic amino acid, immune function, fresh cow

2224 Ruminal and intestinal dry matter and protein degradation of fermented and unfermented pennycress and camelina meals determined using the modified 3-step procedure. S. Wendt*, X. Sun, B. Hu, and I. Salfer, *University Of Minnesota, St. Paul, MN.*

Pennycress and camelina have been investigated as winter-hardy crops that prevent land erosion in the field and can be used to generate oils for renewable biofuel production and resulting meals could potentially be novel protein feeds for livestock. The objective of the study was to examine ruminal and intestinal DM and CP degradability of pennycress and camelina meal without or with fungal fermentation. To accomplish this objective, the modified 3-step assay was performed. Seven oilseed meals were compared including: unfermented pennycress (UP), fermented pennycress meal (FP), unfermented camelina meal (UC), fermented camelina meal (FC), defatted camelina meal (DC), with soybean meal (SM) and canola meal (CAN) controls. An in situ assay was performed using 2 ruminally cannulated cows, with feeds weighed into Dacron polyester bags and digested in the rumen for 2, 4, 8, 12, 16, 24, and 48 h to determine rate of degradation. Resulting residue was digested in pepsin-HCl in vitro for 2 h followed by pancreatin for 24 h. The effect of feedstuff on ruminal dry matter and protein degradability was evaluated using a mixed effects model including the main effects of feed, time and their interaction, and the random effect of cow. Compared with CAN (36.2%) and SM, FP (63.4%) and UP (59.4%) had greater dry matter degradability (DMD) at 2 h (SE = 2.36; $P < 0.001$), whereas UC DMD (29.7%) was lower than soybean meal, but not different from canola. At 16 h, FP had greater DMD (87.5%) than all other treatments (SE = 2.45; $P < 0.01$), whereas FC (57.4%) and UC (61.2%) had a lower DMD ($P < 0.05$) than SM (76.1%) and UP (75.8%), but were not different ($P > 0.10$) from CAN (68.0%) and or DC (64.2%). SM (97.6%) and FP (91.0) had the greatest extent of digestion at 48 h, whereas CAN (84.6%), DC (83.1%), C (81.3%), and FC (80.6%) had the lowest (SE =

2.36; $P < 0.01$). Generally, ruminal degradability of camelina is lower than soybean and canola, whereas pennycress degradability is greater. Results provide nutritive values of these byproduct oilseed meals as protein sources for dairy cows.

Key Words: oilseed meal, cover crops, byproduct feeds

2225 Evaluation of organic dairy calf health traits. A. G. Schultz*, M. M. Cantor¹, B. J. Heins^{2,3}, I. W. Haagen², T. Muratori¹, and C. D. Dechow¹, ¹*Department of Animal Science, Pennsylvania State University, University Park, PA*, ²*Department of Animal Science, University of Minnesota, St. Paul, MN*, ³*West Central Research and Outreach Center, Morris, MN.*

Organic dairy producers face greater challenges in raising calves due to organic antimicrobial regulations compared with their conventional counterparts. This study aims to evaluate relationships among serum total protein (STP), growth in the first 3 mo of life, and survival in organic calves. From June 2024 through January 2025, we sampled calves on 2 organic dairy farms at weekly or biweekly intervals collecting body weight (BW) during the first 2 weeks after birth ($n = 138$ calves), and 1 ($n = 110$), 2 ($n = 88$), and 3 mo ($n = 92$) of age. We collected blood from 47 calves to measure serum total protein (STP). Average daily gain (ADG) from birth to 1, 2, and 3 mo of age was derived and correlated with BW and STP. Growth traits were evaluated with mixed models to estimate the effect of farm with sample date treated as a random effect; STP was evaluated with a linear model that included independent effects of farm and survival. STP was significantly ($P < 0.05$) correlated with BW and ADG at 1 mo. There was no difference between farms for birth weight (38.7 kg versus 39.1 kg), but for all other growth traits farm 1 had significantly ($P < 0.05$) higher performance. The ADG at 1, 2, and 3 mo was 0.76kg/d, 0.89kg/d, and 0.95kg/d for farm 1 and 0.37 kg/d, 0.49 kg/d, and 0.58kg/d for farm 2. Calves were 15.1kg, 28.0kg, and 38.5kg heavier at 1, 2, and 3 mo of age, respectively, at farm 1. Farm 1 (6.28 g/dL) had higher ($P < 0.05$) STP than farm 2 (5.61 g/dL). There was a tendency ($P < 0.10$) for calves that survived to have higher STP (6.28 g/dL) than those that died before the end of the trial (5.58 g/dL). Considering that STP is closely associated with colostrum management, the farm with higher STP had improved growth, and that higher STP improved survival, organic dairy producers should emphasize colostrum delivery to newborn calves.

Key Words: organic, calf, serum total protein

2226 Effect of pH and heat treatment on the solubility of commercial whey protein phospholipid concentrates. A. McKinnon*, J. Huang, and B. Vardhanabhuti, *Division of Food, Nutrition and Exercise Sciences, University of Missouri, Columbia, MO.*

Whey protein phospholipid concentrate (WPPC), a coproduct obtained through the microfiltration of whey, has garnered interest due to its high protein content and potential functional properties. Although whey proteins have been studied extensively, the functional properties of WPPC have not been fully explored. As solubility fundamentally influences other key functionalities, this study investigated the effects of pH and heat treatment on the solubility of 4 commercial WPPC samples (M1–M4) to elucidate their potential in food applications. The solubility profiles of WPPC samples were evaluated under native and heat-treated conditions across pH 3–8. Native samples (1% wt/wt protein) were prepared and allowed to hydrate overnight, whereas heated samples (5% wt/wt protein) underwent heating at 88°C for 10 min. Following equilibration, samples were centrifuged, and solubility was determined

as the percentage ratio of protein content in the supernatant relative to the total protein content. Surface charge characteristics were assessed through zeta potential measurements of unheated samples. Data were evaluated by ANOVA with means separation. Results demonstrated significant pH and source-dependent effects on solubility. All unheated samples exhibited maximum solubility (77.2%–92.4%) at pH 8 ($P < 0.05$) and minimum solubility (30.1%–41.4%) at pH 4. Zeta potential results correlated with solubility trends, showing higher solubility at pH values corresponding to higher net charge. Heating reduced solubility at pH 5–7, with minimum solubility shifted to pH 5. Interestingly, all samples at pH 3 displayed enhanced solubility after heat treatment. Source-dependence was evident, with M3 and M4 exhibiting overall higher solubility ($P < 0.05$). These findings suggest that pH and heat treatment significantly influenced WPPC solubility through alteration of protein charge and potentially structural properties. Such insights can guide the optimization of WPPC functionality in food applications.

2227 Evaluating the impact of heat stress in dairy cows milked with robotic milking system. M. Higgins*, R. Neupane, B. Shrestha, and S. Paudyal, *Texas A&M University, Department of Animal Science, College Station, TX.*

Heat stress poses a significant challenge to the cows but the effect on cows milking under automatic milking systems (AMS) has not been quantified. The objective was to evaluate the impact of heat stress in cows milked with AMS. We analyzed 439,358 individual milking events from approximately 2,000 crossbred cows in a robotic batch milking facility equipped with 22 DeLaval AMS units from July 2023 to September 2024. Cows were housed in 4 pens based on lactation number and age, with milking metrics such as yield, duration, flow rates, and feed intake recorded from the robotic milking system. Temperature-humidity index (THI) data were recorded from a nearby weather station and categorized as presented in Table 1. Results indicate that cows under no heat stress conditions (THI <72) exhibited high milk yield (8.642 ± 0.07 vs. 7.6 ± 0.08 kg/milking), greater feed intake (0.617 ± 0.001 vs. 0.56 ± 0.001 kg/visit), and longer robotic idle times (597.7 ± 2.94 vs. 580.29 ± 5.14 min/day) compared with heat-stressed cows (THI >79). Mean milk flow rates demonstrated a decreasing trend, with the no heat stress group having the highest flow rate for the right front teat (0.95 ± 0.001 kg/min) and the heat stress groups recording lower rates (Table 1), indicating compromised milking efficiency under heat stress. The findings highlight the importance of optimizing barn environments and AMS management strategies to mitigate heat stress effects on dairy cattle. By leveraging data-driven decision making in AMS, dairy farms can enhance sustainability and maintain productivity under varying climatic conditions.

Key Words: dairy, heat stress, robotic milking

2228 Effects of pH and sources on emulsification properties of whey protein phospholipid concentrate. O. Bomgaars*, J. Huang, and B. Vardhanabhuti, *University of Missouri, Columbia, MO.*

With increasing consumer demand for clean label ingredients and sustainable products, the need for innovative utilization of byproducts is at an all-time high. Whey protein phospholipid concentrate (WPPC), a coproduct from whey protein production, contains high protein content and phospholipids, yet remains underutilized despite its potential functionality. This study investigated the effects of pH and protein source variation on the emulsification properties of WPPC. The WPPC samples were obtained from 3 manufacturers (M1, M2, and M3). Oil-in-water (O/W) emulsions (0.5% wt/wt protein, 5% wt/wt oil, pH 3–8) were formed by mixing WPPC solution with oil, followed by homogenization using a laboratory disperser and ultrasonication before final pH adjustment. Emulsification properties were evaluated by measuring mean droplet size, ζ -potential, rheological properties and creaming stability. Data were evaluated by ANOVA with means separation. Regardless of sources, emulsions formed at pH 4 exhibited significantly larger mean droplet diameters ($D_{4,3}$) of 5.94, 1.57, and 1.15 μ m for M1, M2, and M3, respectively. Emulsions formed at other pH values displayed significantly reduced $D_{4,3}$ (0.42 to 0.67 μ m). The ζ -potential values transitioned from 22.1 to 29.7 mV at pH 3 to –29.8 to –40.5 mV at pH 8, with minimal charge potential observed at pH 4. Flow behavior indices (0.67 to 0.92) showed no significant difference among samples ($P > 0.05$). Higher consistency coefficients (K) were observed at pH 4 and 5 relative to other pH ($P < 0.05$). Correlating with droplet sizes and surface charge properties, emulsions at pH 4 were the least stable with M1, M2, and M3 exhibiting phase separation at days 1, 7, and 14, respectively. Emulsions at other pH values were stable without separation through 14 d of storage. These results indicate that WPPC can effectively stabilize O/W emulsions across a broad pH range, except at pH 4.0. The successful formation of stable emulsions suggests potential applications in high-protein beverages or meal replacement shakes as a clean label alternative to conventional emulsifiers.

Key Words: whey protein phospholipid concentrate emulsion pH

2229 Effects of colostrum feeding frequency on passive transfer immunity and growth in Holstein calves. A. Murillo*, K. Lund, M. Clifton, M. Mahdavi-Yekta, D. Kontechy, and A. Ahmadzadeh, *University of Idaho, Moscow, ID.*

Calves receive immunity directly from consuming colostrum. There is information on the relationship between quality of colostrum, calf immunity, and performance. However, it remains uncertain as to whether colostrum feeding frequency affects calf immunity and performance. The objective was to examine the effect of feeding colostrum one time (CF1; n = 27) or 2 times (CF2; n = 28) on growth, and passive transfer

Table 1 (Abstr. 2227). Impact of THI levels on milk yield, consumption, and robotic milking performance¹

THI level	Yield (kg)	Consumed (kg)	Robotic idle time (min)	Mean flow LF (kg/min)	Mean flow LR (kg/min)	Mean flow RF (kg/min)	Mean flow RR (kg/min)	Milk duration (min)
No heat stress (THI<72)	8.642 ± 0.007 ^b	0.617 ± 0.001 ^b	597.688 ± 2.941 ^b	0.939 ± 0.001 ^b	0.944 ± 0.001 ^b	0.946 ± 0.001 ^b	0.939 ± 0.001 ^b	5.752 ± 0.005 ^b
Mild heat stress (67 ≤ THI ≤ 71)	7.744 ± 0.010 ^a	0.549 ± 0.001 ^a	606.222 ± 6.841 ^b	0.902 ± 0.001 ^a	0.898 ± 0.001 ^a	0.906 ± 0.001 ^a	0.888 ± 0.001 ^a	5.490 ± 0.006 ^a
Moderate heat stress (72 ≤ THI ≤ 79)	7.587 ± 0.006 ^d	0.559 ± 0.001 ^d	590.010 ± 3.399 ^{ab}	0.905 ± 0.001 ^d	0.895 ± 0.001 ^d	0.907 ± 0.001 ^d	0.884 ± 0.001 ^d	5.410 ± 0.004 ^d
Severe heat stress (THI>79)	7.639 ± 0.008 ^c	0.554 ± 0.001 ^c	580.285 ± 5.140 ^a	0.915 ± 0.001 ^c	0.906 ± 0.001 ^c	0.917 ± 0.001 ^c	0.896 ± 0.001 ^c	5.339 ± 0.005 ^c

¹LF = left front; LR = left rear; RF = right front; RR = right rear.

immunity (PTI) by measuring serum Brix and total protein (STP). Fifty-five Holstein calves were weighed and assigned randomly into CF1 or CF2. Both groups received 3–4 L of pasteurized high-quality colostrum (Brix >22%) less than 4 h after birth. The CF2 group received an additional 1.5–2 L of colostrum 6–10 h after birth. Blood samples, withers heights (WH), and BW were obtained on d 3 and 7. Also, WH and BW were measured on d 30 and 45 of age. Health status was scored and recorded during the experiment. Serum Brix% and STP were measured using digital refractometers. The differences in Brix%, STP, IgG, WH and BW were analyzed by ANOVA repeated measures. Colostrum frequency treatment did not influence BW, WH, or rate of gain (d 0 to 45). Furthermore, during the first 45 d after birth, there was no mortality, and the morbidity incidence were not different between CF2 and CF1 (17.8 vs. 14.8%). Mean STP and Brix% were affected by day $\times$ group interactions ($P < 0.05$). On d 3, mean STP and Brix were slightly greater ($P < 0.05$) in CF2 than CF1 (STP: 7.5 vs. 7.1 ± 0.15 mg/dL; Brix: 10.9% vs. $10.4 \pm 0.1\%$), respectively. Mean STP and Brix decreased in both groups by d 7, but the rate of decline was greater in CF1 than CF2 (STP: 7.4 vs. 6.5 ± 0.2 mg/dL; Brix: 10.8% vs. 9.8 ± 0.2). Overall estimated IgG concentration on d 3 and 7 were greater in CF2 than CF1 ($P < 0.05$). Although, serum markers for PTI were slightly greater in CF2, the results appear to indicate that 1 or 2 feedings of pasteurized high-quality colostrum have little effect on successful PTI as all 3 indices were greater than critical cutoff points for PTI in both groups. The colostrum frequency treatment did not influence the growth of the calves.

Key Words: colostrum feeding, passive immunity, growth

2230 Effects of a liquid negative DCAD supplement on pre-calving sorting behavior and dry matter intake. A. Simpson¹, B. Juarez¹, T. Forgues¹, A. Mueller², and A. J. Carpenter¹, ¹*Iowa State University, Ames, IA*, ²*Westway Feed Products, The Woodlands, TX*.

The transition period, spanning 3 weeks before and after calving, is critical for cow health, feed intake, and metabolic incidences. This study compared dry matter intake (DMI) and sorting behavior between multiparous Holstein cows ($n = 48$) fed a negative dietary cation-anion difference (DCAD) diet with no liquid feed supplement (CON) and a treatment group receiving a negative DCAD diet with molasses (MOL; RumaWAY LAUNCH, Westway Feeds Products, The Woodlands, TX). Cows were blocked by parity, days in milk (DIM) at dry-off, and projected 305-d actual milk, and were randomized to treatment within block. Cows were enrolled in the trial 28 d before expected calving.

Individual DMI was recorded daily using the Calan Gate system. Samples of the total mixed ration (TMR) and individual cow refusals were collected twice per week during the pre-fresh period for sorting behavior analysis using particle size distribution as measured using the Penn State Particle Separator (PSPS). Samples were frozen immediately after collection and thawed for 24 h before particle size analysis. Data were analyzed using the MIXED procedure of SAS 9.4 with the main effects of diet, parity, time, and the interactions between treatment and time and parity. Precalving DMI was significantly influenced by time ($P < 0.01$), but not affected by dietary treatment, lactation group, or any interactions ($P \geq 0.15$). However, there was an effect of dietary treatment on sorting behavior; CON cows sorted for the bottom sieve and MOL cows sorted against it ($P < 0.01$). Additionally, there was a treatment $\times$ time interaction for the top, middle, and bottom sieve ($P < 0.01$). These results indicate the inclusion of liquid molasses in the pre-fresh period impacts sorting behavior.

Key Words: transition, liquid molasses, sorting

2231 Automatic cow vocalization recognition technology: Applying machine learning for dairy cow voiceprint identification and situational analysis. T. Y. C. Hui and H. I. Chiang*, *Department of Animal Science, National Chung Hsing University, Taichung, Taiwan (ROC)*.

In the livestock industry, the health and performance of dairy cows significantly affect farm profitability. As technology advances, traditional management methods are evolving into smarter systems. Although cow vocalizations are important for expressing emotions and needs, research on their motivations and meanings is still limited. This study explores automatic recognition of cow vocalizations using machine learning techniques. We created a vocalization database by recording sounds in various situations and preprocessing the data. Machine learning algorithms were then used to analyze and identify the vocal characteristics of cows. The results show that the model achieved an accuracy rate of 86.51% and a Kappa value of 0.8349, indicating strong predictive capability and classification consistency. Although some relative error metrics appeared, the model's predictions were still close to actual values. Overall, our study confirms the feasibility of cow vocalization using voiceprint analysis. Analyzing cows' vocal characteristics can improve cattle management efficiency and enhance animal welfare by facilitating timely decisions based on their health and emotional states.

Key Words: cow vocalization, machine learning, animal welfare

Animal Behavior and Well-Being 2: Posters

2300 Effect of providing inadequate space when pair-housing in young dairy origin calves. D. Langenkamp* and J. Gawthrop, *Calf-Care, North Manchester, IN.*

Current literature suggests that pair-housing pre-weaned calves benefits animal welfare and calf growth performance, especially postweaning. When pair-housing, the same amount of space should be provided per calf creating a pen twice the size of individual calf pens. Commercial producers commonly place 2 calves in a pen sized for one calf. The objective of this study was to measure the health and performance impacts of providing inadequate space when pair-housing young dairy origin calves. Fifty-nine crossbred heifer calves 3–7 d of age were used in a randomized clinical trial. Calves were sourced from a sale barn and shipped to a commercial raising facility. Calves were assigned one of 2 treatments: housed individually in a single pen (CON; $n = 37$ calves) or double occupied in a single pen (D; $n = 22$ calves). All calves were housed in commercial pens separated by wire panels with nose-to-nose contact with other calves and bedded with sawdust preweaning. Individual pens measured 101.6 cm by 121.9 cm. Doubled pens measured slightly larger at 121.9 cm by 121.9 cm. Postweaning, all calves were housed in one pen with outside lot access and bedded with sawdust. Least squares means were analyzed using the Microsoft Excel Data Analysis Toolpak (Microsoft, Redmond, WA). The D calves weighed 5.2 kg less at weaning on day 49 (65.9 ± 4.7 kg, 71.1 ± 2.8 kg respectively, $P = 0.03$) and 7.6 kg less postweaning at day 66 (79.0 ± 6.8 kg, 86.6 ± 4.2 kg respectively, $P = 0.03$). There was no difference in average total treatment costs per head ($\$2.99 \pm 1.29$, $\$2.81 \pm 1.02$ respectively, $P \geq 0.91$). There was no mortality for either treatment group. This study indicates that double housing calves without providing adequate space negatively affects calf growth performance preweaning and immediately postweaning.

Key Words: pair housing, housing, calf

2301 Relationship between the level of passive immunity transfer and behavior in dairy calves managed in an automatic feeding system during the rearing period. E. Morales*, L. Espínola, G. Balero, F. Montana, M. Pastorini, and C. Fiol, *Facultad de Veterinaria, Universidad de la República, Libertad, San José, Uruguay.*

The aim was to characterize the behavior of Holstein and Jersey calves concerning passive immunity transfer (TPI) in an automatic feeding system (AFS) during the preweaning period. Forty-eight calves (BW = 33.78 ± 0.81 kg), born between April and June 2024, were used. Colostrum feeding was natural, with calves remaining with their mothers for the first 12 h, and TPI was evaluated 48 h after separation using refractometry (% Brix). Based on TPI, calves were classified into 3 groups: poor ($<8.1\%$ Brix), acceptable ($8.1\%–9.5\%$ Brix), and excellent ($\geq 9.5\%$ Brix). Between 3 and 5 d of age, calves were introduced to the AFS, with average milk intake of 4.56 ± 0.08 L, concentrate intake of 0.48 ± 0.02 kg, and water and hay offered ad libitum. Behavior was recorded by direct visual scan sampling every 5 min for 3 h in the morning (0900–1200 h) and 3 h in the afternoon (1300–1600 h) over 2 consecutive days every 15 d (4 periods). Two postures (standing/lying) and 9 activities were recorded: eating concentrate, eating fiber (ef), ruminating standing, ruminating lying down (rld), walking, drinking milk, drinking water, licking standing (ls), and licking lying down. Data were analyzed using PROC GLM of SAS On Demand, considering TPI level, period, and their interaction as fixed effects. An effect of TPI level ($P < 0.01$)

was observed on lying time, where calves with excellent TPI spent more time lying than those with poor or acceptable TPI (0.32 ± 0.02 vs. 0.22 ± 0.02 vs. 0.18 ± 0.02 , respectively). TPI also affected drinking milk, with calves with excellent TPI spending more time in this activity (0.09 ± 0.01 vs. 0.04 ± 0.01 vs. 0.03 ± 0.01 ; $P < 0.01$). Additionally, calves with poor TPI spent more time rld than those with acceptable TPI (0.09 ± 0.01 vs. 0.04 ± 0.01 ; $P < 0.01$). Passive immunity transfer also influenced ls and ef, where calves with excellent TPI showed more time in these behaviors than calves with poor TPI (0.02 ± 0.004 vs. 0.01 ± 0.004 to ls and 0.04 ± 0.01 vs. 0.02 ± 0.01 to ef; $P < 0.01$). In conclusion, TPI affects calf behavior during the preweaning period, especially in time spent lying or standing, feeding, and ruminating.

Key Words: automatic feeder, behavior, dairy calf

2302 Effect of serotonin on milk quality, production, and dam activity in a cow-calf contact system. N. N. Teixeira*, B. J. Meyer, L. R. Lewandowski, S. J. J. Adcock, and L. L. Hernandez, *University of Wisconsin–Madison, Madison, WI.*

On dairy farms, calves are typically separated from their dams soon after birth, which can negatively impact animal welfare. Cow-calf contact systems can improve welfare but may reduce milk yield and quality and increase fatigue in the dam. We aimed to determine whether administering prepartum 5-hydroxytryptophan (5HTP), which elevates blood serotonin levels, could increase the dam's milk quality, milk yield, and activity (an inverse proxy for fatigue) in a cow-calf system. We assigned 23 pregnant Holstein cows to receive 5HTP ($n = 12$) or saline ($n = 11$) administered once daily in a single infusion beginning 10 d before calving. Cows receiving 5HTP or saline for 4 to 14 d were eligible for the study. After calving, the cow-calf pairs were housed in individual cow-calf pens for 30 d using the following gradual weaning protocol: d 0–7, cows and calves were kept together 24 h a day (24H); d 8–21, cows and calves were kept together for 12 h at night and separated for 12 h during the day (12H) where they could see but not touch each other, and; d 22–30, where they were separated for 24 h a day, but could see each other. Cows were milked twice daily. Activity and lying time were recorded from 12 to 2 pm on d 1, 3, 7, 14, and 21 using triaxial accelerometers placed on the cow's right hind leg. Mixed linear models were used for analysis, with the effects of treatment, day, and their interaction as fixed effects and cow as a random effect. No effect of treatment or treatment by time interactions was observed for milk production ($P = 0.46$; $P = 0.99$, respectively) or somatic cell count (SCC; $P = 0.13$; $P = 0.64$, respectively). Milk production increased and SCC decreased with DIM ($P < 0.001$). Overall activity ($P = 0.97$) and lying time ($P = 0.01$) did not differ across days but cows that received 5HTP prepartum tended to be more active than those receiving saline ($P = 0.08$). No treatment effect on lying time was observed ($P = 0.22$). In conclusion, prepartum 5HTP tended to increase the cow's activity, potentially reflecting reduced fatigue, but lying time as well as milk yield and quality were unaffected.

Key Words: welfare, 5-hydroxytryptophan (5HTP), milk production

2303 Effects of preweaning social housing of dairy calves on long-term performance and first-lactation milk yield. E. E. Lindner*, J. Bonney-King, and E. K. Miller-Cushon, *University of Florida, Gainesville, FL.*

Prewaning social housing of dairy calves is known to provide short-term benefits for performance and animal welfare, yet there are gaps in knowledge surrounding longer-term production implications. The objective of this study was to assess the effects of preweaning social housing on age and body weight at first calving and milk yield during the first lactation. We analyzed data of cows previously raised individually (IH; $n = 60$), in pairs (PH; $n = 60$), or in groups of 10 (GH; $n = 54$) until 9 weeks of age. Included animals had complete lactation records until 100 DIM. Age at first calving did not differ between previous housing treatments (699 d; SE = 5.6; $P = 0.6$) but body weight at calving was greater for PH than IH, with GH intermediate (GH = 603.4, PH = 619.4, IH = 600.0 kg; SE = 5.7; $P = 0.033$). Milk yield data were analyzed in linear mixed models including fixed effects of treatment, season of calving (warm: April to September, cool: October to March), week of lactation as a repeated measure, and BW at calving as a covariate. Milk yield was greater for PH and GH than IH (GH = 38.6, PH = 38.3, IH = 36.6 kg/d; SE = 0.6; $P = 0.045$). Milk yield was also greater overall in the cool season (+7.0 kg/d; SE = 1.5; $P < 0.001$) and subject to a marginal interaction between treatment and calving season ($P = 0.10$) where differences between treatments were most apparent for cows that calved in the cool season (60% of enrolled animals; +2.7 kg/d for GH and +3.4 kg/d for PH vs. IH; SE = 1.1; $P = 0.005$) but not significant within the warm season (40% of enrolled animals; $P = 0.47$). These results suggest long-term advantages in milk yield for cows reared in 2 different forms of social housing, compared with cows reared individually during the preweaning period.

Key Words: social housing, milk yield, season

2304 The effect of maternal bovine appeasing substance administration at dry-off on eating, rumination, and activity behavior in Holstein dairy cows. M. Ruch^{*1}, E. Shepley¹, A. Pereira¹, J. Matiello¹, E. Bonsaglia^{1,2}, T. Scheler¹, E. Leonard¹, and L. Caixeta¹, ¹University of Minnesota, St. Paul, MN, ²University of São Paulo, Pirassununga, São Paulo, Brazil.

Maternal bovine appeasing substance (MBAS) has emerged as a tool to help mitigate stress in cattle, but limited data exists on its application in dairy cows around stressful management periods. This cluster randomized controlled study evaluated the effect of administering a MBAS (FerAppease, Fera Diagnostics and Biologicals) at dry-off on dairy cow behavior (total daily rumination, eating, and activity time) in the first 14 d following dry-off. Holstein cows were enrolled on one Minnesota farm at dry-off into 2 groups: a treatment group (TRT; $n = 100$), receiving 7.5 mL of MBAS topically behind the poll and above the muzzle, and a control group (CON; $n = 93$). Treatment was applied at the pen level to prevent contact between CON cows and the MBAS applied to TRT cows. Cows were randomly assigned to a treatment, determining their pen placement, and treatment assignments alternated with each new enrollment, ensuring a minimum 3-week washout period to prevent contact between groups. Behavior data were recorded using neck-mounted sensors (AfiCollar, AfiMilk Ltd.). Linear mixed effects models were used to analyze the behavioral data, including the fixed effect of treatment, day relative to enrollment, parity, baseline behavioral data (rumination, eating, and activity data from 7 d before dry-off), pen allocation, and relevant interactions. Cow was considered a random effect. Compared with CON cows, TRT cows had higher total daily rumination time across the first 7 d (416 vs. 384 min/d; $P < 0.01$) and 14 d (429 vs. 402 min/d; $P = 0.01$) following dry-off, with the most noticeable difference between d 3 to 6 after dry-off. Total time spent eating was also higher in TRT cows across the first 7 d (278 vs. 261 min/d; $P = 0.02$) and 14 d after dry-off (304 vs. 285 min/d; $P = 0.04$)

compared with CON cows. Activity was not affected by treatment ($P > 0.05$). These results indicate that MBAS application at dry-off increases rumination and eating time, suggesting improved stress adaptation.

Key Words: stress management, transition management, pheromone treatment

2305 Infrared temperature accuracy measured at 2 anatomical sites on dairy calves. M. L. Pister^{*} and J. K. Drackley, University of Illinois at Urbana-Champaign, Urbana, IL.

We aimed to compare skin temperatures measured with infrared thermometers to rectal temperatures (RT). The experiment included 20 Holstein bull calves, enrolled at 16–18 d of age, and housed individually in hutches with straw bedding. Feeding and medical treatments were conducted per research protocol. Infrared temperatures (IRT) and RT were recorded daily at 0700 and 1800 h. Anatomical regions used were neck and rectal surface. The IRT measurements were taken from a 30 cm distance, with 2 readings recorded from each region and all 4 averaged for analysis. Heart rate (HR) and respiratory rate (RR) were recorded daily, whereas ambient temperature and relative humidity were monitored hourly by on-site data loggers. Data were analyzed using the MIXED and CORR procedures in SAS. Variables used to assess IRT accuracy included RT, environmental heat stress (EHS), and hutch heat stress (HHS). Heat stress (HS) was defined as temperature-humidity index (THI) ≥ 79 . A weak correlation was observed between RT and neck IRT ($r = 0.11$), and between RT and rectal surface IRT ($r = 0.13$). However, the IRT ranges differed (neck: 8.8–60°C; rectal surface: 4.3–54.4°C), which could be attributed to factors such as time of day, direct sunlight, and hair color. In contrast, RT was maintained closely by the calf (37.6–40°C), making the relationship difficult to apply on-farm without further analysis. No correlation was observed between average daily RT and EHS; there was a weak correlation between RT taken at 1800 h and EHS ($r = 0.21$) and HHS ($r = 0.12$). No correlation was observed between IRT and EHS or HHS for either region. Calves experiencing HHS had lower neck IRT ($P = 0.03$) and HR ($P < 0.001$) compared with non-heat stressed (NHS) calves. This may be attributed to calves preferring time outside when hutch THI is high, where weather conditions could aid in cooling. Moreover, HS may affect lying behavior, with calves altering posture to maximize heat loss. No differences were observed in RR between HS and NHS. The IRT provided statistically significant relationships, but did not accurately reflect RT with the parameters measured.

Key Words: infrared temperature, heat stress, calf

2306 Dairy consumer attitudes toward a hypothetical animal welfare initiative. J. Robbins¹, K. Proudfoot², L. Hemsworth³, G. Coleman³, P. Hemsworth³, and J. Van Os^{*1}, ¹Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI, ²Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI, Canada, ³Animal Welfare Science Centre, University of Melbourne, Parkville, VIC, Australia.

The dairy industry has developed corporate social responsibility (CSR) initiatives to demonstrate their commitment to animal welfare. However, little is known about how US dairy consumers perceive these initiatives. Previous research on CSR initiatives in other contexts suggests that the extent to which they are perceived as driven by strategic (e.g., to promote consumer confidence in dairy) or altruistic (e.g., to improve animal welfare) affects consumers' attitudes. Our objective was to assess how US dairy consumers perceived the motivations of a fictitious

dairy company initiative focused on improving on-farm human-animal interactions, and to evaluate how these related to their attitudes toward the initiative and company. In an online survey, participants (n = 344 who reported consuming dairy at least once per week) read a fictitious news article about an animal welfare CSR initiative, then provided ratings of motivational attributions (whether they thought the initiative was strategic or altruistic, 1 = strongly disagree; 7 = strongly agree) and their attitudes toward the initiative and the company (1 = more negative, 7 = more positive). Participants were 50.6 ± 16.9 (mean ± SD) years old, 52.9% female, 77.3% white, and 32.3% college graduates. Spearman rank correlations were used to evaluate associations between motivational attributions and attitudes toward the initiative and the company. General attitudes were positive toward the initiative (5.4 ± 1.3) and company (5.3 ± 1.2). The initiative was perceived as both strategic (4.9 ± 1.4) and altruistic (5.6 ± 1.2), and these motivational attributions were correlated ($\rho = 0.25$). More positive attitudes toward the initiative and company were associated more strongly with more altruistic ($\rho = 0.56$ and 0.68 , respectively) vs. strategic ($\rho = 0.22$ and 0.26) motivational attributions. We found that motivational attributions are a key factor in dairy consumers' evaluations of animal welfare CSR efforts, with altruistic attributions more strongly determining their attitudes compared with strategic ones. To improve the impact of animal welfare CSR initiatives, future research could investigate how these motivational attributions are formed.

Key Words: consumer perception, public trust, corporate social responsibility

2307 Do light intensity and temperature influence abnormal grouping behavior of Holstein cows during the summer in northern New York? A. L. Bartlett*, A. E. Pape, S. Y. Morrison, and C. S. Ballard, *William H. Miner Agricultural Research Institute, Chazy, NY*.

A retrospective study was performed to investigate if light intensity and/or temperature differ within a pen during crowding events. Lactating Holstein cows (n = 88, 141 ± 50.2 DIM) housed in a north-south oriented 4-row freestall barn were evenly split into 2 adjacent pens of similar size and layout for replication. Cows were milked 3× per day and fed 1× per day. Abnormal grouping behavior (AGB) was evaluated by determining spatial preference via video footage scanned every 10 min between 0900 h and 1900 h daily from June 15 to July 8, 2024. Pens were visually split into 4 sections where sections 1 (S1) and 4 (S4) contained crossovers and adjacent alleys (~72 m²) and sections 2 (S2) and 3 (S3) contained stalls and alleys (~171 m²). Available space per cow in each pen section was calculated every 10 min based on the number of cows within that section. Abnormal grouping behavior was considered when available space per cow was ≤6.0 m². Crowding events (CE) were identified as consecutive (≥1) 10 min intervals of AGB (n = 76 CE). Light intensity (lux) and air temperature (°C) were measured continuously in each pen section at 10 min intervals. Data were analyzed with GLM procedure of SAS v. 9.4 with fixed effects of CE and pen section. Tukey procedure for multiple comparison was used when pen

section was significant. For both pens, CE only occurred in S1. When cows were crowding in S1, the light intensity was lowest relative to the other sections of the pen, likely because S1 contained solid exterior barn walls with no open curtains to allow natural light into the barn. Section 2 had intermediary light intensity with the next highest cow density. Although not biologically significant, air temperature was highest in S1 (Table 1). The small elevation in temperature may be attributed to less air movement through this section of the barn or the body heat generated from large cow numbers in a small area.

Key Words: crowding event, light intensity, temperature

2308 The impact of delaying cow-calf separation on dairy cow behavior. K. R. Callero*, G. M. Frederick, T. A. Westhoff, and S. Mann, *Cornell University, Ithaca, NY*.

Recent welfare concerns from the public have brought the practice of immediate cow-calf separation into question, however, there is a lack of research observing the behavior effects of delaying cow-calf separation. Thus, the objective was to describe cow behavior when calf separation was delayed in the first week postpartum. We conducted a block randomized controlled trial with treatments stratified by parity (primiparous vs. multiparous). Holstein dairy cows were allowed to maintain contact with their newborn calf for 0 (0D; n = 6), 3 (3D; n = 5), 5 (5D; n = 6), or 7 d (7D; n = 6). After parturition, all enrolled cow-calf pairs were moved from the maternity pen into identical individual box stalls with an attached calf pen and remained there for 8 d of observation during which they were milked 2×/d. Non-contact groups were physically separated by a solid barrier. Continuous audio and video recording and measurement of activity with cow leg sensors was performed. Heart rate monitors were placed on each cow for 4 h after morning milking on the day before and the day of separation. Video data were analyzed in a subset of 16 cows (0D, n = 4; 3D, n = 3; 5D, n = 4; 7D, n = 5). Behavioral Observation Research Interactive Software (BORIS) was used to code footage of enrolled cows 24 h before and 24 h after separation. Heart rate data were analyzed in MATLAB and activity monitor data were analyzed in SAS. All other data manipulation was performed in Excel. All cows who had contact with their calves did not reject them, spent time grooming them and there were no calf injuries observed. Cows in the 5D and 7D groups had increased number of vocalizations ($P = 0.006$) after separation as compared with those in the 0D and 3D groups. The 5D group also had a higher difference in heart rate standard deviations ($P = 0.04$) than other groups. By observing cows and their calves together for 3, 5, or 7 d, we described aspects of a cow's behavioral response to delaying separation. Our results suggest that cows have a measurable behavior response to delayed separation, and additional research is necessary to understand possible influences on health and welfare.

Key Words: behavior, separation, welfare

2309 Computer vision analysis of dairy calf behavior under different heat abatement and feeding strategies during a subtropical

Table 1 (Abstr. 2307). Characteristics of pen sections during crowding events

Item	Pen section				SE	P-value	
	S1	S2	S3	S4		CE	Section
Mean space per cow ± SD, m ²	5.1 ± 0.7	10.2 ± 1.7	38.3 ± 28.6	34.9 ± 18.8	—	—	—
Light, lx	937 ^c	1,600 ^b	2,108 ^a	2,013 ^a	80	<0.01	<0.01
Temperature, °C	26.35 ^a	26.26 ^b	26.21 ^c	26.30 ^b	0.01	<0.01	<0.01

^{a-c}Differing superscripts within rows significantly different $P \leq 0.05$.

summer. C. Saha^{*1}, C. G. Savegnago¹, Y. B. Amancio¹, R. M. da Costa¹, J. Gao¹, A. M. Roper¹, T. N. Marins¹, N. Andriotti¹, M. C. Guenther², A. Amato³, J. Laporta², S. Tao¹, and A. A. C. Alves^{1,4}, ¹University of Georgia, Athens, GA, ²University of Wisconsin, Madison, WI, ³Università degli Studi di Messina, Messina, Sicily, Italy, ⁴Institute for Integrated Precision Agriculture, Athens, GA.

This study aimed to explore the use of computer vision systems (CVS) to assess individual calf behavior under different heat abatement and feeding strategies. This research was performed on a commercial dairy farm in Southern Georgia for 6 d in early July 2024. Heat abatement included positive pressure tube ventilation (PPTV) at 1.5 m/s wind speed and daytime misters (CL) or no PPTV (HT). Calves were assigned to 2 feed treatments: low milk intake (LMI; 4 L) or high milk intake (HMI; 9 L). These factors were arranged in a 2 × 2 factorial design creating 4 unique treatment groups. Thirteen 4-MP cameras captured images every 10 s, monitoring 39 hutch-housed calves (14 ± 3.5 d of age) in 3 shaded areas. The YOLOV7 algorithm was trained to detect different calf behaviors using 2,000 manually annotated images, captured on late June. This dataset was split as 90% for training and 10% for validation, achieving on average 92% precision, 89.6% recall, and 92.2% mean average precision (mAP) for validation images. Using the trained YOLOV7 model, we analyzed 6 d of testing images (early July) to measure the time calves spent standing, lying down, feeding grain, drinking water, and drinking milk, generating 5,073 hourly observations per behavior class. A Bayesian mixed model was used to analyze treatment effects on each behavior across 3 periods: morning, afternoon, and night. The posterior distribution for treatment contrasts (*pBayes*) indicates strong evidence that HMI calves spent more time lying down than LMI calves in the afternoon and night (*pBayes* < 0.03), whereas LMI calves stood more (0.003 < *pBayes* < 0.027) in these periods. Moderate evidence suggests LMI calves spent more time eating grains and drinking water (*pBayes* < 0.09). Notably, CL calves spent more time lying down in regions closer to the tubes compared with HT calves, especially in the afternoon (12 min on average), suggesting calves actively seek ventilation during summer. Our results show that CVS are a valuable tool for automatic, noninvasive calf monitoring, supporting efficient heat stress abatement strategies.

Key Words: heat stress, ventilation, YOLOV7

2310 The effect of abnormal grouping behavior on behavior and performance of Holstein cows during the summer in northern New York. A. L. Bartlett^{*}, A. E. Pape, S. Y. Morrison, and C. S. Ballard, William H. Miner Agricultural Research Institute, Chazy, NY.

Bunching is an abnormal grouping behavior (AGB) that indoor-housed cows can exhibit during the summer. The effects of AGB on other behaviors and production are unknown. A retrospective study was performed from June 15 to July 8, 2024, to determine if AGB participation impacts cow behavior or performance. High-producing lactating Holstein cows [*n* = 88; 141 ± 50.2 d in milk (DIM); 50.6 ± 7.1 kg/d milk yield; mean ± SD] housed in a 4-row, sand-bedded freestall barn were evenly split into 2 adjacent pens of similar size and layout for replication. Cows were milked three times per day and fed once per day. Pens were balanced by lactation number, DIM, body weight, BCS, and milk production. Abnormal grouping behavior was evaluated by determining spatial preference via video footage scanned every 10-min daily. Crowding events (CE) were identified as ≥1 consecutive 10 min intervals of AGB between 0900 and 1900 h daily (*n* = 74). Continuous location data were obtained for a focal group of cows (*n* = 71) to determine CE participation (i.e., location and time within the CE was confirmed). Milk yield, water intake, reticular temperature, rumination, and lying behavior were evalu-

Table 1 (Abstr. 2310). Coefficients of linear mixed regression of selected variables against minutes of abnormal grouping behavior by day

Item	β	P-value
Milk yield, kg/d	0.0033	0.03
Water intake, L/d	-0.028	0.001
Median reticular temperature, °C	0.00007	0.5
Rumination, min/d	0.089	0.004
Lying time, min/d	-0.56	<0.001
Lying events/d	-0.0023	0.01
Lying time/rumination, min/min	-0.0011	<0.001

ated by day. Data were analyzed as linear regressions with the MIXED procedure of SAS version 9.4 with fixed effect of minutes of AGB and random effect of cow. On days where bunching was observed, event duration ranged from 10 to 230 min/d. Total AGB participation ranged from 0 to 360 min/cow per day. The estimated regression coefficients are shown in Table 1. The strongest effect was on lying time, such that for every additional hour of participation, 34 min of lying time was lost per participating cow per day. Participating cows did not make up for lost lying time and slightly increased rumination time while standing. Future research should focus on methods of reducing bunching behavior during the summer.

Key Words: crowding event, lying time, behavior

2311 Effects of caustic paste application method on wound healing in dairy calves. S. Yoo^{*}, M. A. G. von Keyserlingk, and D. M. Weary, The University of British Columbia, Vancouver, BC, Canada.

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

Caustic paste disbudding is known to require a longer healing time than hot-iron disbudding. The aim of this study was to determine if healing time could be reduced by changes in paste application method. Twenty Holstein heifer calves were disbudded at 4 to 6 d of age. Calves were paired based on birth order, and within each pair, one calf was randomly assigned to receive a larger caustic paste application area (LARGE: 22 mm diameter), whereas the other received a smaller area (SMALL: 12 mm diameter). Within each calf, caustic paste thickness was randomly assigned to each horn (THIN: 1 mm vs. THICK: 2 mm). Before disbudding all calves received multimodal pain relief, including sedation, a cornual nerve block, and a nonsteroidal anti-inflammatory drug. To ensure uniform application, metal circle stencils were used to control both the paste thickness (1 mm or 2 mm) and the application area (12 mm or 22 mm in diameter). The horn buds were positioned at the hole in the center of the stencils, and paste was applied and leveled using a spatula. Wound size and depth were measured weekly using a digital caliper while the calves were restrained. All data, except scour occurrence, were analyzed using a mixed model with fixed effects of paste thickness, application area, and their interaction, with calf included as a random effect. Horn buds in the LARGE and THICK treatments received more caustic paste (mean ± SE, SMALL: 0.15 ± 0.02 g vs. LARGE: 0.39 ± 0.02 g; THIN: 0.22 ± 0.02 g vs. THICK: 0.32 ± 0.02 g; both *P* < 0.001), resulting in larger wounds (mm²; SMALL: 178.2 ± 19.1 vs. LARGE: 494.7 ± 19.1; THIN: 316.7 ± 14.7 vs. THICK: 356.2 ± 14.7; *P* < 0.001 and *P* = 0.003, respectively). Necrotic tissue detached earlier in the SMALL and THIN treatments (weeks, SMALL: 7.3 ± 0.83 vs. LARGE: 13.2 ± 0.88; THIN: 9.43 ± 0.69 vs. THICK: 11.1 ± 0.69; *P* < 0.001 and *P* = 0.02, respectively), and wounds in these groups healed more quickly (weeks, SMALL: 12.7 ± 1.02 vs. LARGE: 18.6 ± 1.07;

THIN: 15.0 ± 0.79 vs. THICK: 16.3 ± 0.79 ; $P < 0.001$ and $P = 0.04$, respectively). Horn scurs were observed in all SMALL-treated horns (18/18) and none in the LARGE-treated horns (14/14). In conclusion, both the application area and thickness of the caustic paste influenced healing time. Thinner application reduced damaged area and healing times. A smaller application area had even greater positive effects on tissue damage and healing, but the treatment level we tested (12-mm circle) was insufficient to fully prevent horn growth.

Key Words: welfare, chemical burn, tissue damage

2312 Disbudding dairy calves with caustic paste: Assessing the effect of contact duration on the healing process. A. M. Drwencke^{*1}, S. J. J. Adcock², and C. B. Tucker¹, ¹University of California, Davis, Davis, CA, ²University of Wisconsin, Madison, Madison, WI.

Caustic paste disbudding has grown in popularity on US dairy farms. The methods of paste application vary across the literature and effect wound healing. Our objective was to determine whether the duration of paste contact with the horn bud effected rubbing paste off by the calf, wound size, healing, and regrowth. At 3 d of age, 30 Holstein calves were randomly assigned to 1 of 2 treatments ($n = 15$ each). Calves were disbudded with a "Normal" method, where 0.2 mL of Dr. Naylor's paste was applied to horn buds shaved with a 40-mm blade and left to dry, or "Wiped," where the paste was removed after 1 h. The prevalence of paste rubbing was observed 1 h and 4 h (Normal calves only) after application. Wound diameter was measured 1 d after disbudding and 5 wk later. We evaluated the presence and absence of 8 tissue types and horn regrowth once or twice per week for 21 wk. Using a generalized linear mixed model, paste rubbing showed no evidence of differing by treatment (23% vs. 30% of horn buds for Wiped and Normal, respectively, $P = 0.65$). A linear mixed model was used to assess wound diameter with treatment, side, time, and the interaction between time and treatment as fixed effects and calf as a random effect. Rubbing was included as a covariate and did not affect wound size or healing time ($P \geq 0.34$). Wound diameter was similar between treatments ($P = 0.12$); wounds were (mean $\pm$ SE) 18.9 ± 0.5 mm and 20.1 ± 0.8 mm 1 d after disbudding, and 5 wk later they increased to 23.0 ± 0.8 mm and 22.7 ± 0.6 mm for Normal and Wiped treatments, respectively. The probability of advancing to 3 tissue types (crust, epithelium, fully healed) was evaluated using a survival analysis. There was no evidence that treatment affected the probability of advancing to the next healing stage for the 3 tissue types (63% vs. 73% crust, 47% vs. 57% epithelium, and 40% vs. 33% fully healed for Normal vs. Wiped; $P \geq 0.30$). Using a Fisher's exact test, Wiped horn buds tended to have more horn regrowth ($P = 0.07$) and removal of the regrowth ($P = 0.09$) compared with the Normal treatment. Wiping paste after 1 h was not beneficial for paste rubbing, wound size, or healing, and tended to increase the risk of regrowth.

Key Words: pain, burn, management

2313 Using milking robot data for genetic evaluation of behavioral traits in Holstein cattle. H. Bérat^{*2,1}, N. Gengler², J. M. Maskal¹, J. P. Boerman¹, and L. F. Brito¹, ¹Department of Animal Sciences, Purdue University, West Lafayette, IN, ²Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium.

The adoption of automated milking systems (AMS) in modern dairy farming has expanded the possibilities for data-driven genetic studies. AMS provide detailed records of cow performance and behavior, enabling new traits to be derived for genetic selection. This study explored the genetic basis of AMS-derived behavioral traits in American

Holstein cattle by assessing their phenotypic variability during lactation and estimating variance components and genetic parameters. Data were analyzed from 5,645 cows, collected in 36 AMS units from 2018 to 2021. Seven behavioral traits were examined: average milking time (AMT), total milking time (TMT), interval between milkings (INT), number of visits to AMS units (NoV), number of successful entries (NSE), percentage of successful milkings (PSM) and preference consistency score (PCS). Variance components and heritability estimates were calculated using a repeatability model with 2 different approaches to modeling the permanent environment: between parities and both between and within parities. Heritability estimates revealed genetic variability ranging from low (PSM: 0.05–0.06; NoV: 0.10) to moderate (TMT: 0.27–0.32; INT: 0.08–0.10) and high (AMT: 0.46–0.48). The PCS, which quantifies cow preferences for specific AMS units, showed heritability ranging from 0.09 to 0.24 depending on model structure. Genetic correlations revealed favorable associations, such as between INT and PSM (0.64–0.71), suggesting that optimizing milking intervals could improve success rates. Conversely, negative correlations, such as between PSM and NoV (–0.93), highlighted potential tradeoffs between frequent visits and milking efficiency. Traits such as AMT and INT showed consistent genetic control and strong potential as indicators of milking adaptation. These results show that the potential integration of such traits into selection programs could be considered. Future studies will focus on random regression models to assess trait dynamics during lactation, and by investigating the genomic regions underlying these traits to promote sustainable dairy farming practices.

Key Words: automated milking system, behavioral genetics, genetic parameter

2314 Evaluating the effects of heat stress on cows milked with robotic milking systems using machine vision algorithms. R. Neupane^{*1}, B. Shrestha¹, S. Flake², M. Mohan², S. Chaudhary², C. Daigle¹, and S. Paudyal¹, ¹Department of Animal Science, Texas A&M University, College Station, TX, ²Department of Computer Science and Engineering, Texas A&M University, College Station, TX.

The objective was to evaluate the impact of heat stress on the behavior of cows milked with robotic milking systems using machine vision algorithms. Hourly temperature and humidity data for the location was obtained from the nearest weather station. A freestall pen housing 240 milking cows with 3 robotic milking units at a dairy farm in central Texas was monitored. An RGB video monitoring camera setup (GWS Security, South El Monte, CA) recorded cow activity from 6:00 a.m. to 8:00 p.m. daily from May 2024 to July 2024. The region of interest (ROI) was defined as the holding area in the perimeter of the robots with dimensions (13.71 m length $\times$ 13.4 m width). An image processing pipeline was developed using OpenCV and Os Python modules to extract the ROI every minute with a total of 42,291 images extracted. We randomly selected a total of 271 images for labeling using RoboFlow, capturing varying lighting conditions and time periods. Additionally, image pre-processing (resizing and contrast adjustment) and augmentation (flip, and rotation) were used to generate additional training images (3X). The dataset was annotated into 3 classes: busy robot, idle robot, and cows in the robotic milking area. A YOLOv11 model for object detection was trained, which achieved a precision of 85.54%, a recall of 84.16%, a mAP@50 of 88.36%, and a mAP@50–95 of 53.99%, indicating strong detection performance with challenges in precise localization at higher IoU (intersection over union) thresholds. The relationship between the temperature-humidity index (THI) categories and the number of cows in the robotic milking area was analyzed using an ordinary least squares (OLS) regression model. Results indicated a significant effect of THI,

with a higher presence of cows (14.57 ± 0.09 vs. 12.8 ± 0.08 vs. 11.40 ± 0.07 ; $P < 0.01$) in the holding area under low THI (<72), medium THI (72–79) and high THI (>79) respectively. Future research will focus on identifying individual robot occupancy, calculating robotic

idle time, and evaluating operational milking efficiency under varying heat stress conditions.

Key Words: computer vision, dairy, heat stress

Animal Health 2: Posters

2315 Effects of systemic inflammation in postpartum dairy cattle on inflammatory mediators during an intramammary *Streptococcus uberis* challenge. A. Celemin Sarmiento^{*1}, M. Bulnes-Larsen¹, S. Dilberger-Lawson², C. Chase², and T. H. Swartz¹, ¹*Department of Dairy and Food Science, South Dakota State University, Brookings, SD*, ²*Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD*.

Elevated concentrations of proinflammatory cytokines during the peripartum period have been associated with an increased risk for postpartum diseases including mastitis in dairy cattle. Nevertheless, the direct effects of systemic inflammation on mammary gland immune responses have yet to be elucidated. Therefore, the objective of this study was to evaluate the effects of recombinant bovine tumor necrosis factor α (rbTNF α) on inflammatory mediators in postpartum dairy cattle during an intramammary challenge. Parous Holstein dry cows were blocked by parity, expected calving date, and genetic merit for milk yield (PTAM) and randomly assigned within block to receive a daily SC injection of either (1) rbTNF α (n = 10; 2 μ g/kg BW in 10 mL PBS) or (2) saline (CON, n = 10; 10 mL of PBS) from d 1 to 7 postpartum. All cows received an intramammary *Streptococcus uberis* challenge (2,500 cfu) in a randomly selected rear quarter at 4 d postpartum. Blood (d 1 to 8) and milk samples from the challenged quarter (d 4 to 8) were collected once daily. A multiplex bovine cytokine kit was used to quantify IL-6, IL-10, MIP-1 α , MIP-1 β , and TNF- α in plasma and milk serum. Data were analyzed using linear mixed models (PROC GLIMMIX in SAS) with the fixed effects of treatment, time, and their interaction and the random effects of cow and block. Plasma ($P < 0.0001$) and milk TNF α ($P = 0.05$) were greater in rbTNF α as compared with CON. Plasma MIP-1 α ($P < 0.01$) concentrations were greater in rbTNF α as compared with CON. There was a treatment $\times$ time interaction ($P \leq 0.05$) for plasma IL-6, IL-10, and MIP-1 β . Plasma IL-6 concentrations were greater on d 1 ($P < 0.001$) for rbTNF α , but no treatment effects were observed afterward. Plasma IL-10 and MIP-1 β concentrations were greater in rbTNF α on d 1 through 7 ($P < 0.001$). Treatment did not affect milk concentrations of IL-6 ($P = 0.86$), IL-10 ($P = 0.89$), MIP-1 α ($P = 0.73$), or MIP-1 β ($P = 0.88$). In conclusion, induction of systemic inflammation via rbTNF α induced a broad systemic cytokine response; however, aside from enhancing milk TNF α , rbTNF α did not affect mammary gland cytokine responses.

Key Words: cytokine, postpartum dairy cow, mastitis

2316 Efficacy of a nanotechnology-based dry cow therapy during experimentally induced *Escherichia coli* mastitis after dry-off. J. Gao^{*1}, C. G. Savegnago¹, T. N. Marins¹, A. M. Roper¹, Y. B. Amancio¹, N. Andriotti¹, T. Barrows², and S. Tao¹, ¹*Department of Animal and Dairy Science, University of Georgia, Athens, GA*, ²*Cure-Mast Inc., Atlanta, GA*.

Cubosomes are discrete, cubic liquid crystalline phase, amphiphilic lipid nanoparticles featuring 2 interpenetrating hydrophilic regions separated by lipid bilayers. We previously showed that intramammary (i.m.) infusion of an emulsion of cubosomes recruited immune cells into the mammary gland. We aimed to evaluate the efficacy of 2 prototypes (FT1 and FT2) cubosomes as a potential dry cow therapy during *Escherichia coli*-induced mastitis after dry-off (DO). Both FT were made from the same lipid ingredients with varied processes (CureMast Inc., Atlanta, GA). Four healthy late-lactation Holstein cows (quarter milk

SCC < 200,000 cells/mL, no presence of bacteria) were enrolled before DO. After the last milking, quarters within each cow were alternatively infused with 10 mL of FT1, FT2, and saline (SAL, 2 quarters). At 24 h post-DO, 10 mL of *E. coli* 727 suspension (360 cfu/mL) was i.m. infused into quarters that received FT1, FT2 and SAL, while the remaining SAL quarter did not receive *E. coli* and served as a control (CTL). Quarter milk or mammary secretion (MS) samples were collected every 12 h from -48 to 264 h relative to DO for composition analysis, and *E. coli* enumeration was performed at 36, 48, 72, 96, 120, 144, and 168 h post-DO. Data were analyzed using PROC MIXED procedures of SAS. Before *E. coli* challenge, i.m. infusion of FT2 increased ($P \leq 0.05$) MS SCC at 12 and 24 h post-DO relative to FT1 and SAL. After *E. coli* challenge, MS SCC was greater ($P \leq 0.01$) in FT1+*E. coli*, FT2+*E. coli* and SAL+*E. coli* quarters than CTL quarter at 36, 48, 60, 96 and 120 h post-DO. Treatments had no effect on MS fat, protein, or lactose contents ($P \geq 0.56$). The *E. coli* counts in MS from FT1+*E. coli* and SAL+*E. coli* quarters peaked (17,000 cfu/mL) at 48 h after DO then declined to 120 cfu/mL at 120 h ($P < 0.01$). MS collected from FT2+*E. coli* quarter had no *E. coli* grown throughout the study. Cryo-transmission electron microscopy suggested FT2 was more effective than FT1 because of its higher content of cubosomes. In conclusion, cubosomes represent a promising new dry cow therapy for preventing bacterial infection in the early dry period.

Key Words: cubosome, dry-off, mastitis

2317 Epigenetic response to heat stress in dairy cattle: Insights from blood methylome analysis. G. Costa Monteiro Moreira^{*1,2}, A. Ruiz-González³, M. Joigner⁴, V. Costes⁵, A. Chaulot-Talmon^{1,2}, F. Ali^{1,2}, H. Kiefer^{1,2}, H. Jammes^{1,2}, and D. E. Rico⁶, ¹*BREED, Université Paris Saclay, UVSQ, INRAE, Domaine de Vilvert, Jouy-en-Josas, France*, ²*Ecole Nationale Vétérinaire d'Alfort, BREED, 94700, Maisons-Alfort, France*, ³*De'partement des Sciences Animales, Université Laval, Québec, QC, Canada*, ⁴*Idele, Paris, France*, ⁵*Eliance, Paris, France*, ⁶*Centre de Recherche en Sciences Animales de Deschambault (CRSAD), Deschambault, QC, Canada*.

Epigenetics plays a crucial role in livestock adaptation to environmental challenges like heat stress. Despite its crucial role, there is still a lack of information about the epigenetic mechanisms involved in dairy cow adaptation/response to heat stress. Here we aim at identifying heat stress-induced DNA methylation variations on blood methylome potentially affecting regulatory regions and associated phenotypes. Multiparous Holstein cows (42.2 ± 5.6 kg milk/d; 83 ± 27 DIM) were housed in a climate-controlled tie-stall barn with automated temperature regulation. During the 14-d experiment, temperature followed a daily cycle: 29°C to 39°C (0800–1400 h), 39°C (1400–1700 h), 39°C to 29°C (1700–2300 h), and 29°C overnight (2300–0800 h). The temperature-humidity index (THI) ranged from 72–82 (heat stress) to 61–64 (thermoneutral). Blood samples were collected and peripheral blood mononuclear cell (PBMC) isolated from 4 cows before and after heat stress (d 0, d 14) and from 4 cows in thermoneutral conditions (d 0, d 14). Reduced representation bisulphite sequencing (RRBS) was used to profile PBMC's methylome. Differential methylation analysis was performed using *methyKit* and *DSS* software (Ameth $\geq 25\%$, adjusted P -value < 0.01), retaining only commonly detected differentially methylated cytosines (DMCs). A total of 4,104 DMCs were identified when comparing pre- and post-heat stress samples. After excluding 1,195 DMCs also found in thermoneutral conditions, 2,909 heat stress-specific DMCs remained, predominantly

hypomethylated. Approximately half of the DMCs are annotated in intronic and intergenic regions; known to harbor regulatory elements. By intersecting the DMRs with publicly available functional annotation data, we observed hypomethylation on regulatory regions putatively affecting cow's immune system. As an example, a hypomethylated DMR in an alternative transcript of *ARHGEF6* gene was identified, suggesting an impact on isoform usage. The Rac/Cdc42 guanine nucleotide exchange factor 6 (*ARHGEF6*) gene has its expression enhanced in immune cells (granulocytes) and exerts crucial biological functions in T cell migration, consequently affecting the immune system. *ARHGEF6* was also previously associated with milk protein yield and somatic cell score in dairy cattle. These findings highlight the impact of heat stress on dairy cow immunity and provide new insights into epigenetic regulation under environmental stress.

Key Words: heat stress, epigenetics, methylation

2318 Balancing precision and practicality: The use of MALDI-TOF in dairy cow mastitis diagnostic. L. da Costa^{*1}, A. Della Libera², B. Nascimento de Araujo³, and J. C. Ribeiro Jr.⁴, ¹The Ohio State University, Columbus, OH, ²Federal University of Vale of São Francisco, Petrolina, PE, Brazil, ³Federal University of Northern Tocantins, Tocantins, TO, Brazil, ⁴Eastern Laboratory Service, Medina, OH.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) is essential for rapidly and accurately identifying bacteria in milk samples, which is crucial for timely mastitis treatment. By identifying pathogens, veterinarians can customize treatments, reduce antibiotic use, and improve animal health and milk quality. However, the challenge lies in determining which of the many identified bacteria are clinically relevant and require treatment. Thus, the aim of this study is to provide the prevalence of the main pathogens causing mastitis in dairy cows, thereby enabling informed decisions to improve mastitis management. Results were compiled from the analysis of 34,298 bulk milk tanks from the Midwest United States from November 2023 to July 2024. A total of 273 different pathogens were identified, and the more prevalent or commonly causing mastitis pathogens are presented. *Staphylococcus* spp. was among the most frequently identified organism. A descriptive analysis by pathogen shows the number of samples identified, the average prevalence (%), and the range for each of the organisms. The most prevalent organism was *Staphylococcus chromogenes* with 3,441 samples (average prevalence of 10%; range 9%–12%). Second was *Staphylococcus aureus* with 2,868 samples (7.7%; range 7%–9%), and *Streptococcus uberis* with 2,554 samples (7.4%; range 5%–9%). *Streptococcus dysgalactiae* had 1,891 samples, and a prevalence of 5.7% (range 4%–8%). *Lactococcus* species included *L. garviae* had 961 samples (2.8%; range 2%–4%), and *L. lactis* had 1,014 samples (3%; range 2%–4%). Interestingly, *Escherichia coli* was found in 977 samples in 2.9% of the tanks (range 2%–3%), and *Klebsiella* spp. was found in less than 1% of the tanks (*Klebsiella pneumoniae*, 0.6% [range 0.4%–1%] and *Klebsiella oxytoca*, 0.6% [range 0.3%–1%]). *Aerococcus viridans* was found in 2,178 samples, with a prevalence of 6.6% (range 4%–9%). Although it has occasionally been isolated from subclinical mastitis infections in dairy cows, its prevalence is still unknown. Although bulk tank milk samples are less sensitive than individual quarter-level samples, understanding pathogen prevalence aids in making informed herd management and mastitis prevention decisions.

Key Words: mastitis, dairy cow, MALDI-TOF diagnostic

2319 Antimicrobial potential and genomic characterization of *Bacillus* spp. isolated from gauze collected from teat skin of dairy

cows against *Staphylococcus aureus*. E. Bonsaglia^{*1,2}, F. Peña-Mosca³, V. Rall⁴, M. Santos², K. Lang¹, N. Noyes¹, and L. Caixeta¹, ¹University of Minnesota, St. Paul, MN, ²University of São Paulo, Pirassununga, São Paulo, Brazil, ³Cornell University, Ithaca, NY, ⁴Sao Paulo State University, Botucatu, São Paulo, Brazil.

Bovine mastitis remains one of the major challenges in dairy production, causing significant economic and productive impacts. Disease control still relies heavily on the use of antibiotics, and their indiscriminate use contributes to the development of antimicrobial resistance in production systems. New approaches to bacterial control are needed. The objective of this study was to isolate *Bacillus* spp. and evaluate their ability to inhibit *Staphylococcus aureus*. To achieve this, we isolated sporeforming bacteria from the teat skin of dairy cows and assessed their antimicrobial activity through phenotypic and genomic analyses. Ten gauze samples used for cleaning the teat skin of dairy cows on an organic farm were placed in PBS and heated in a water bath at 80°C for 15 min. These samples were plated on Luria-Bertani (LB) agar and incubated at 37°C for 24 h (n = 10). From each plate, 16 colonies were selected and purified. To evaluate the ability of each isolate to inhibit *S. aureus*, the strains were cultivated in LB broth and subsequently plated on tryptic soy agar (TSA). After 24 h of incubation, the plates were inverted, and a culture of *S. aureus* ATCC 25923 was spread on the agar surface. Following an additional 24 h of incubation, the plates were expected for the presence of zones of inhibition where *S. aureus* growth was inhibited. Among the 160 isolates evaluated, 33% (n = 53) exhibited inhibitory activity, as qualitatively determined by the presence of zones of inhibition. The statistical analysis used in this study was descriptive. The isolate with the highest antimicrobial potential was subjected to whole genome sequencing and identified as *Bacillus licheniformis*. Genome analysis using the antiSMASH software revealed the presence of biosynthetic gene clusters (BGCs) involved in the production of secondary metabolites with antimicrobial potential. Specifically, our analyses identified predicted BGCs related to the synthesis of the secondary metabolites lichenysin, lichenicidin, and bacillibactin. Additionally, the presence of clusters with low similarity to known biosynthetic pathways suggests the potential discovery of novel bioactive compounds or structural variants. These findings indicate that *B. licheniformis* may represent a promising source for the development of new antimicrobial agents against *S. aureus*.

Key Words: antimicrobial resistance, bovine mastitis, sporeforming bacteria

2320 Unveiling transcriptomic insights into matrine's mechanism against *Streptococcus agalactiae* in dairy mastitis. Y. R. Zhao, H. Zhang, and J. J. Tong^{*}, Beijing University of Agriculture, Beijing, China.

Mastitis caused by *Streptococcus agalactiae* (GBS) leads to significant economic losses in the dairy industry. Matrine, a natural alkaloid from *Sophora* species, has demonstrated antimicrobial, immunomodulatory, and anti-inflammatory properties, but its specific mechanism against GBS remains unclear. This study aimed to elucidate the transcriptomic and functional impact of matrine on GBS virulence factors. All experiments were performed with 3 independent biological replicates for each treatment group. GBS cultures were treated with matrine at 0, 25, 50, and 100 µg/mL, and bacterial growth, adhesion, biofilm formation, and capsular polysaccharide production were assessed. We performed RNA sequencing on triplicate GBS samples from control and matrine-treated groups, followed by differential gene expression analysis and functional enrichment analysis. We used qRT-PCR to validate key transcriptomic findings. Statistical analyses were conducted, including one-way ANOVA

and Student's *t*-tests, with results expressed as mean $\pm$ SD. Matrine significantly reduced GBS adhesion ($47.6 \pm 2.3\%$, $P < 0.01$), survival rates ($31.2 \pm 1.7\%$, $P < 0.05$), capsular polysaccharide production ($58.4 \pm 3.1\%$, $P < 0.01$), and biofilm formation ($52.8 \pm 2.8\%$, $P < 0.01$) in a concentration-dependent manner. Transcriptomic analysis identified 135 differentially expressed genes (DEGs) in matrine-treated GBS, with significant downregulation of virulence-associated genes related to adhesion (*fbxA*, *scpB*), capsular biosynthesis (*cpsE*, *cpsL*), and biofilm regulation (*luxS*, *htrA*). These findings suggest that matrine disrupts GBS pathogenicity by impairing immune evasion and invasion mechanisms. This study provides novel insights into matrine's antimicrobial mechanism and highlights its potential as an antibiotic-free therapeutic alternative for GBS-induced mastitis in dairy cattle.

Key Words: matrine, *Streptococcus agalactiae*, transcriptomic analysis

2321 Evaluating the combined impact of early genomic predictions for mastitis and somatic cell score on udder health in Holstein Cows.

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Mastitis remains the most prevalent and costly disease in dairy cattle. Understanding the relationship between genomic traits and udder health may aid in developing strategies to mitigate its negative impact. This study evaluated the association between genomic resistance to mastitis (GZMAS) and genomic prediction of somatic cell score (GSCS) with somatic cell count (SCC) and clinical/subclinical mastitis parameters. We hypothesize that cows with high resistance to mastitis and low GSCS would have fewer subclinical and clinical mastitis cases than those with low resistance and high GSCS throughout strategic decision windows in the lactation cycle. Data were collected from 4,509 genotyped Holsteins across 3 California Central Valley herds using DairyComp305. Cows included in the analysis were at least 200 DIM and had genomic health data. The GZMAS and GSCS traits were categorized into terciles (T1 = lowest, T3 = highest). For data presentation purposes, results were highlighted for 2 extreme groups: low resistance/high SCC (T1T3) and high resistance/low SCC (T3T1). Data were analyzed using mixed models in JMP (SAS). Herds that had similar bulk tank SCC and incidence of mastitis were used as a random effect in the models. Cows with high resistance/low SCC had significantly lower SCC (T3T1 = 72.3 ± 18.8 cells/mL vs. T1T3 = 146.1 ± 14.1 cells/mL, $P = 0.001$) and lower subclinical mastitis incidence at previous lactation dry-off (T3T1 = $6.7 \pm 1.2\%$ vs. T1T3 = $15.4 \pm 1.1\%$, $P = 0.001$). Similarly, at the first test date, high resistance/low SCC cows had reduced SCC (T3T1 = 103.6 ± 21.5 cells/mL vs. T1T3 = 238.1 ± 20.5 cells/mL, $P = 0.001$) and lower subclinical mastitis incidence (T3T1 = $8.5\% \pm 1.2\%$ vs. T1T3 = $15.7\% \pm 1.2\%$, $P = 0.001$). By 150 DIM, these cows had lower clinical mastitis incidence (T3T1 = $5.6 \pm 1.1\%$ vs. T1T3 = $19.2 \pm 1.0\%$, $P = 0.001$), fewer clinical cases per cow (T3T1 = 0.11 ± 0.01 vs. T1T3 = 0.27 ± 0.01 , $P = 0.001$), and fewer subclinical cases per cow (T3T1 = 0.58 ± 0.06 vs. T1T3 = 1.54 ± 0.05 , $P = 0.02$). These findings suggest that integrating genomic data on mastitis resistance and somatic cell score can guide mastitis management and treatment strategies in Holstein cows.

Key Words: subclinical, somatic cell score, mastitis

2322 Assessing the associations of the precalving udder health with genomic predictions for mastitis and somatic cell score in

Holstein heifers. M. C. G. M. Silva^{*1}, T. J. Almand¹, A. Ueda¹, M. J. Wollentarski¹, N. Fragata¹, N. Machado¹, B. Melody², P. Bos², R. Wood², E. Regusci², D. Bruno³, and F. S. Lima¹, ¹Department of Population Health and Reproduction, University of California, Davis, Davis, CA, ²Lander Veterinary Clinic Inc., Turlock, CA, ³Cooperative Extension, University of California Agriculture and Natural Resources, Fresno, Fresno, CA.

Mastitis in dairy heifers remains a significant challenge on dairy farms, and research on the utility of genomic resistance to mastitis (GZMAS) and genomic predisposition for high somatic cell count (GSCS) in guiding precalving antibiotic use is limited. This study evaluated the association between GZMAS and GSCS with culture and PCR results for mastitis pathogens at 30 d precalving. Data were collected from 404 genotyped Holsteins from a commercial dairy farm in California. Heifers included in the analysis were at least 245 d of gestation and had genomic health data. The GZMAS and GSCS traits were categorized into terciles (T1 = lowest, T3 = highest). For data presentation purposes, results were highlighted for 2 extreme groups: low resistance/high SCC (T1T3, $n = 93$) and high resistance/low SCC (T3T1, $n = 135$). For GZMAS (T1 ≤ 97 ; T3 ≥ 103) and GSCS (T1 ≤ 2.6 ; T3 ≥ 2.8). Milk secretion samples were aseptically collected from each quarter by the research team, placed immediately on ice, and sent to the milk quality laboratory at Lander Veterinary Clinic. Samples were screened by culture and PCR for *Mycoplasma bovis*, *Mycoplasma californicum*, *Mycoplasma canadense*, *Mycoplasma bovigenitalium*, *Mycoplasma alkalescens*, *Staphylococcus aureus*, and *Streptococcus agalactiae*. Data were analyzed using mixed models in JMP (SAS). Heifers classified as high resistance/low SCC had a lower incidence of culture-positive cases (T3T1 = $45.0\% \pm 5.1\%$ vs. T1T3 = $72.5\% \pm 5.8\%$, $P = 0.001$), fewer culture-positive cases with multiple affected quarters (T3T1 = $17.1\% \pm 10.9\%$ vs. T1T3 = $45.5\% \pm 10.4\%$, $P < 0.001$), and fewer affected quarters per heifer (T3T1 = 1.0 ± 0.3 vs. T1T3 = 1.8 ± 0.3 , $P = 0.001$). Similarly, high resistance/low SCC heifers tended to have fewer PCR-positive cases (T3T1 = $3.4\% \pm 2.0\%$ vs. T1T3 = $6.2\% \pm 4.0\%$, $P = 0.06$) and a lower incidence of culture-positive *Staphylococcus aureus* (T3T1 = $5.2\% \pm 5.0\%$ vs. T1T3 = $11.9\% \pm 5.4\%$, $P = 0.08$). Precalving heifers classified as high resistance/low SCC based on their genomic profile exhibited improved udder health before entering their first lactation. These findings suggest that genomic information can be a valuable tool in guiding precalving targeted therapies.

Key Words: heifer, treatment, genetics

2323 Distribution of mastitis-causing bacteria in cases of sub-clinical mastitis from low bulk tank somatic cell count dairy herds.

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The objective of this study was to estimate the relative frequency of mastitis-causing bacteria (MCB) from cows with subclinical mastitis (SM) on low bulk tank somatic cell count (SCC) Wisconsin dairy herds. Herds with bulk tank average SCC from the processor for the last 12 mo $\leq 150,000$ cells/mL were eligible for enrollment. Five farm visits were conducted in 2024; where >40 cows with high SCC ($\geq 200,000$ cells/mL) were sampled. Two sets of quarter milk samples, one aseptic and one non-aseptic, from each eligible cow were collected. Aseptic samples were transported to the University of Wisconsin–River Falls milk quality laboratory to be frozen until analysis. Non-aseptic samples were preserved with bronopol and transported to a commercial laboratory for SCC analysis, as only aseptic samples with $\geq 200,000$ cell/mL were used for microbiological analysis. We used MALDI-TOF MS for

Table 1 (Abstr. 2323). Microbiological diagnoses

Microbiological diagnosis	Number diagnosed	%
No bacterial growth	78	46.2
<i>Enterococcus saccharolyticus</i>	24	14.2
Mixed infection	17	10.1
<i>Staphylococcus simulans</i>	9	5.3
<i>Staphylococcus chromogenes</i>	8	4.7
Other (gram-positives)	7	4.1
<i>Lactococcus garvieae</i>	6	3.6
<i>Staphylococcus epidermidis</i>	6	3.6
<i>Streptococcus uberis</i>	3	1.8
<i>Staphylococcus aureus</i>	3	1.8
Yeast	3	1.8
<i>Staphylococcus species</i>	2	1.2
<i>Enterococcus faecium</i>	1	0.6
<i>Lactococcus lactis</i>	1	0.6
<i>Klebsiella pneumoniae</i>	1	0.6
Contaminated	35	17.2

bacterial speciation. A total of 536 quarter milk samples were collected, and of those milk samples, 204 were cultured for further microbiological analysis. Microbiological diagnoses are depicted in Table 1. Based on our preliminary data, the distribution of the main etiologies for subclinical cases differed from other studies. The 3 most prevalent bacterial groups included no growth (no bacteria isolated), non-aureus staphylococci, and *Enterococcus spp.* In conclusion, it is important to use microbiological tests that allow for bacterial speciation as phenotypic tests would not provide an accurate bacterial identification leading to misdiagnoses.

Key Words: subclinical mastitis bacteria

2324 Automated detection and classification of digital dermatitis in dairy cattle using computer vision and YOLOv11 algorithms. R. Neupane*¹, B. Shrestha¹, P. Pinedo², and S. Paudyal¹, ¹Department of Animal Science, Texas A&M University, College Station, TX, ²Department of Animal Sciences, Colorado State University, Fort Collins, CO.

The objective was to develop a system capable of detecting and classifying digital dermatitis (DD) lesions in dairy cattle using computer vision models to achieve early diagnosis and reduce subjective human scoring. Cows with DD were identified at the milking parlor and marked for evaluation at the trimming chute, where hoof images were captured using a red-green-blue (RGB) camera (GoPro Hero 9) from a distance of 1 foot, focusing on the interdigital area where DD lesions are common. The dataset contained images of 280 individual cows with DD lesions, manually scored by an experienced observer from early (D1) to chronic (D4.1) stages, which were classified as D1 (early, small <2 cm lesion), D2 (ulcerative or granulomatous lesion >2 cm), D3 (healing stage with scab-like covering), D4 (chronic, dyskeratotic lesion with thickened epithelium or surface proliferation), and D4.1 (healing stage lesion). The lesion distribution was 7% (D1), 22% (D2), 30% (D3), 24% (D4), and 17% (D4.1). Image preprocessing (resizing, auto-orient, and contrast adjustment) and augmentation (horizontal flip, crop, and exposure) were performed using RoboFlow, and the dataset was split into training (88%), validation (8%), and testing (4%) sets. Two YOLOv11 models were trained: an object detection model, achieving 0.8506 precision, 0.7593 recall, mAP@50 (mean average precision at 50% intersection over union) of 0.8119, and mAP@[0.50:0.95] (mean average precision

at intersection over union thresholds from 50% to 95%) of 0.3415, indicating robust segmentation and detection; and a lesion classification model, which used a Python pipeline with OpenCV and OS modules to extract regions of interest (ROIs), achieving 0.77 weighted accuracy, 0.83 precision, 0.81 recall, and 0.80 F1-score, excelling in detecting early (D1) and severe (D4) lesions. We successfully developed and validated a computer vision-based system for detecting and classifying DD lesions with acceptable accuracy, and future research will focus on improving localization accuracy, deploying models on edge devices (e.g., NVIDIA Jetson Nano, Raspberry Pi) for on-farm use, and integrating predictive analytics for lesion progression tracking.

Key Words: computer vision, digital dermatitis, dairy

2325 Effects of blackberry pomace-derived phenolic compounds on bovine mammary epithelial cells survival rate. A. Grif-fin*¹, H. Eldaous^{1,2}, S. Rajput¹, C. O. Lemley¹, X. Zhang¹, and J. Halfen¹, ¹Mississippi State University, Mississippi State, MS, ²Benha University, Mushtuhur, Toukh, Qalioubia, Egypt.

This study examines the effect of blackberry pomace-derived phenolic compounds (PC) on bovine mammary epithelial cell (MAC-T) survival. The PC were extracted from dried, ground blackberries, and their total antioxidant capacity was measured using the Folin-Ciocalteu method. The MAC-T cells, cultured in 75-cm² flasks to 85% confluency, were plated at 100,000 cells/mL in a clear 96-well plate, and incubated for 24 h at 37°C with 5% CO₂. Cells were incubated with or without PC for 0, 6, 12, or 24 h, and treatments included control (media only), 5, 10, 20, 50, and 100 mg/mL PC. Cell survival was quantified using the Cell Proliferation Kit I (MTT; Roche, Germany). Data were analyzed using the MIXED procedure of SAS, including a total of 4 replicates, with survival rate expressed as % over the control. Overall treatment (trt; $P < 0.0001$) effects were observed for greater survival rate in cells treated with 100 mg/mL (265.43% $\pm$ 8.0%) in comparison with 50 mg/mL PC ($P < 0.05$, 172.72% $\pm$ 7.8%), 20 mg/mL PC ($P < 0.05$, 124.72% $\pm$ 7.8%), 10 mg/mL PC ($P < 0.05$, 101.32% $\pm$ 7.8%), 5 mg/mL PC ($P < 0.05$, 113.73% $\pm$ 0.04%), and control ($P < 0.05$, 107.22% $\pm$ 7.8). There was a trt $\times$ time interaction ($P < 0.0001$) among treatments at 6 h, 12 h, and 24 h, resulting in highest survival rate for cells treated with 100 mg/mL in all time points compared with the other treatments ($P < 0.05$). No differences between treatments (trt $\times$ time, $P > 0.05$) were observed at 0 h. At 6 h and 12 h, the 50 mg/mL treatment also increased cell survival compared with 20 mg/mL, 10 mg/mL, 5 mg/mL, and control ($P < 0.05$), but remained lower than 100 mg/mL PC ($P < 0.05$). The 100 mg/mL and 50 mg/mL PC treatments showed the highest survival rate at 12 h (324.60% $\pm$ 16.5% and 236.11% $\pm$ 15.0%, respectively), followed by a decline at 24 h (294.78% $\pm$ 13.2% and 139.03% $\pm$ 13.2%, respectively), suggesting an optimal response with 12 h incubation. These findings indicate that blackberry pomace-derived PC can increase cell survival in a time and dose-dependent manner.

Key Words: blackberry, cell survival, phenolic compound

2326 Dairy cow health in cow-calf contact systems on commercial farms in North America. M. Durrenwachter*¹, E. Nogues¹, J. H. C. Costa¹, D. L. Renaud², and K. Creutzinger¹, ¹University of Vermont, Burlington, VT, ²University of Guelph, Guelph, Ontario, Canada.

Separation of dairy calves from the dam soon after birth has become a contentious issue in the dairy industry. However, there is a lack of research to investigate cow health and production on commercial dairy farms using cow-calf contact systems to rear calves. This observational

study assessed welfare, health, and milk quality of dairy cows rearing their calves on 12 farms in the United States ($n = 10$) and Canada ($n = 2$). Farms were eligible for inclusion if dam-calf contact was the primary calf rearing system; they were visited 3 times in spring, summer, and fall 2024. Body condition score (BCS), hygiene, lameness, and chaps on teat skin were scored for all cows nursing their calves. A total of 430 cows were assessed (mean $\pm$ standard deviation: 36 ± 27 cows per farm) and were 59.5 ± 58.8 DIM (range: 1–306 d). Cows nursing their calves and less than 30 d in milk (DIM) had milk samples collected (287/430 cows) to estimate nonesterified fatty acid (NEFA), β -hydroxybutyrate (BHB), and somatic cell count (SCC; cells/mL). Descriptive results are presented. No cows were emaciated (BCS < 2) and 7% were considered over-conditioned (BCS ≥ 4). Seven percent of cows were severely lame, and few cows were scored as “dirty” (4% of cows). Teat chaps were present on at least 1 teat for 2% of the cows. Somatic cell count was below $< 200,000$ cells/mL for 62% of cows, 9% were between 200,000 to 400,000 cells/mL, 2% had levels between 400 and $750 \times 1,000$ cells/mL and 21% of cows had SCC above the limit of saleable milk in the United States ($750 \times 1,000$ cells/mL). Milk concentration of NEFA indicative of negative energy balance (> 0.6 mEq/L) was present in 15% of the cows, whereas no cows had BHB above the cut-point indicative of subclinical ketosis (> 1.2 mmol/L; 0.10 ± 0.08 mmol/L). These results indicate that dairy cows rearing their calves are within industry benchmarks; although most variables were at expected level, further investigation on intramammary health and energy balance is warranted.

Key Words: cow-calf contact, welfare, milk composition

2327 Association of maternal experience and morbidity with daughter performance in dairy cows. A. Akbar¹, A. Husnain¹, A. Rehman¹, Q. Shahid², H. Rahman², M. Ahmed¹, T. Khan¹, Z. Hassan¹, T. Ashraf¹, A. Riaz¹, and Z. Sarwar^{*3}, ¹Department of Theriogenology, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan, ²Department of Livestock Production, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan, ³Department of Animal Sciences, University of Florida, Gainesville, FL.

A retrospective cohort study evaluated the data of 216 Holstein heifers born from 2016 to 2023 at the university research farm. The objectives were to determine the association of maternal experience and morbidity with the performance during the heifer stage and first lactation through 300 DIM. Maternal experience was defined as follows: heifers born from the first calving were classified as P1, from the second calving as P2, and the third or greater calving as P3+. Maternal morbidity was defined as follows: heifers born from dams that experienced clinical disease after calving but before pregnancy establishment were classified as HMorbD, otherwise, they were classified as HNoMorbD. Further, if the heifer had clinical disease during the first 120 d of age, it was classified as CMorb120, otherwise, CNoMorb120. The body weight (BW), health, and reproduction during the heifer stage and health, production, and reproduction during the first lactation were assessed. Data were analyzed by MIXED, GLIMMIX, and PHREG procedures in SAS. The heifer born from P1 and P2 dams had higher ($P < 0.01$) BW from 11 to 17 mo of age as compared with the heifers from P3+ dams. Similarly, heifers born from younger dams (P1 or P2) had higher ($P = 0.05$) pregnancy (79.2% vs. 86.0% vs. $63.5\% \pm 7.4\%$) and became pregnant faster during the heifer stage than P3+ dams. The HMorbD had lower ($P < 0.01$) BW than HNoMorbD, which likely explained the lower ($P < 0.01$) hazard of pregnancy during the heifer stage. However, maternal experience and morbidity had no association with calf morbidity, monthly or 305-d milk yield and reproduction during the first lactation. The CMorb120 was associated with higher ($P = 0.03$) morbidity

by 300 DIM (36.1% vs. $72.2\% \pm 12.1\%$), cows leaving the herd (6.5% vs. $22.2\% \pm 6.9\%$), and low ($P = 0.03$) 21-d cycle pregnancy rate and hazard of pregnancy, which resulted in extended days open during the first lactation. However, calf morbidity had no association with BW and reproduction during the heifer stage and production during the first lactation. In short, dam morbidity is associated with compromised heifer growth and reproduction, and heifers from younger dams perform better. Calf morbidity increases the risk of clinical disease, reducing survival and reproduction during the first lactation.

Key Words: maternal experience and morbidity, health and growth, production and reproduction

2328 The effect of dietary supplementation of *Bacillus subtilis* PB6 to mid-lactation dairy cows on neutrophil response following ex vivo bacterial stimulation. H. L. Reisinger^{*1}, O. Reyes¹, L. K. Mamedova¹, I. E. Brown-Crowder², J. E. Hergenreder², T. H. Swartz³, and B. J. Bradford¹, ¹Department of Animal Science, Michigan State University, East Lansing, MI, ²Kemin Industries Inc., Des Moines, IA, ³Department of Dairy and Food Science, South Dakota State University, Brookings, SD.

Dietary supplementation of probiotics may be an effective way to support cow health and enhance immune function to fight off pathogens. Neutrophils, one of the first immune cell responders to an infection, have a major role in pathogen clearance by phagocytosis. Our objective was to evaluate the effects of dietary *Bacillus subtilis* PB6 supplementation on phagocytosis and oxidative burst of neutrophils following an ex vivo whole-blood bacterial stimulation. Mid-lactation Holstein cows ($n = 20$; 153 ± 48 DIM, 42.1 ± 7.6 kg/d milk) were enrolled in a crossover design. Treatments were *B. subtilis* PB6 (STAT; CLO-STAT, Kemin Industries, Inc.; 13 g/d), or limestone (CON; 13 g/d) top-dressed onto the diet immediately following feed delivery. Blood was collected on d 22–23 of each 28-d period and whole blood (100 μ L) was stimulated with either lyophilized pHrodo *Escherichia coli* or *Staphylococcus aureus* bioparticles in the presence of dihydrorhodamine 123 (DHR) for 30 min (39°C , 5% CO_2). Following incubation, bioparticle and DHR fluorescence were measured by flow cytometry to determine bacterial uptake and oxidative burst of neutrophils. Fixed effects included treatment, period, and bovine leukemia virus status and random effects included cow and block. The total proportion of phagocytosing neutrophils was unaffected by treatment regardless of stimulation (*E. coli*: 76.0% STAT vs. 75.5% CON, $P = 0.85$; *S. aureus*: 73.7% STAT vs. 76.9% CON; $P = 0.17$). Furthermore, the proportions of fully activated neutrophils, defined as neutrophils performing both phagocytosis and oxidative burst, were not affected by treatment for either stimulation (*E. coli*: 68.6% STAT vs. 69.6% CON, $P = 0.70$; *S. aureus*: 66.2% STAT vs. 69.6% CON; $P = 0.14$). Similarly, treatment had no effect on the bioparticle median fluorescence intensity (MFI) of fully activated neutrophils, which is a proxy for bioparticle uptake per neutrophil. Overall, *B. subtilis* PB6 supplementation to mid-lactation cows for 22–23 d does not appear to improve bacterial phagocytosis or oxidative burst in an ex vivo whole-blood stimulation.

Key Words: direct-fed microbial, nutritional immunology

2329 Exploring the role of inflammatory microRNAs in bovine colostrum. J. Peres Prietsch^{*1,2}, R. Santos¹, C. Cassal Brauner^{2,3}, E. Schmitt^{2,3}, and F. Rosa¹, ¹School of Veterinary Medicine, Texas Tech University, Amarillo, TX, ²Center for Research, Education, and Extension in Livestock (NUPEEC), Federal University of Pelotas, Pelotas,

RS, Brazil, ³Department of Animal Sciences, Federal University of Pelotas, Pelotas, RS, Brazil.

Colostrum is a bioactive secretion produced by the mammary gland, rich in immunoglobulins, growth factors, and cytokines, which are essential for neonatal metabolic and immune development. Recent studies have identified microRNAs (miRNAs) in colostrum, highlighting their key role in gene regulation, immune modulation, cell differentiation, and apoptosis. This study aimed to evaluate the presence of inflammatory related miRNAs in the bovine colostrum. Colostrum samples (50 mL) were collected from multiparous Holstein cows ($n = 10$) within 4 h after calving for total RNA extraction, miRNA enrichment, and miRNA quantification using RT-qPCR with specific miRNA primers. Among the miRNAs detected, let-7a-5p was the most abundant across samples (mean CT = 22), followed by miR-21-5p, miR-101-3p, and miR-29c-3p (mean CT = 25). However, miR-142, miR-222-3p, and miR-340-3p were low expressed (mean CT = 30). MicroRNAs let-7a-5p and miR-21-5p are associated with immune regulation during intramammary infections, whereas the miR-29 family is involved in different lactation pathways. Additionally, miR-101-3p may play a role in calf development, potentially influencing neonatal immunity and metabolic programming. In contrast, miR-142 is a potential biomarker of mammary gland health. Further studies are required to elucidate the regulatory pathways of colostrum-derived miRNAs and their implications for early life programming, immune development, and dairy production. Understanding these mechanisms could contribute to optimizing colostrum and milk quality in commercial dairy systems and improving neonatal and mammary gland health.

Key Words: cattle, colostrum, noncoding RNAs

2330 Incidence of BLV infection on commercial dairy farms in Ontario, Canada. O. Willoughby^{*1}, E. I. Morrison¹, F. van der Meer², D. Kelton¹, K. L. Spence¹, and S. J. LeBlanc¹, ¹Department of Population Medicine, University of Guelph, Guelph, ON, Canada, ²Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada.

Bovine leukemia virus (BLV) is a production limiting disease of dairy cattle. Infection with BLV is associated with reduced production efficiency and a higher likelihood of premature culling. Despite successful eradication of BLV in several countries, the prevalence of BLV in North America has continued to increase over time. To control the spread of BLV and prevent new infections, a better understanding of how BLV is transmitted is required. Therefore, this study aimed to quantify and describe the incidence of BLV infections in commercial dairy settings. In total, 6,424 Holstein dairy animals >6 mo of age were sampled on 10 commercial dairy farms in Ontario Canada. Farms ranged in size from approximately 150 to 500 milking animals. Farms were pre-selected for the study based on previous bulk tank BLV results, and had initial BLV prevalence estimates ranging from 23% to 59% based on test-day records. For individual testing, serum ELISA was used to measure antibodies to BLV; seropositivity was defined as $\geq 45\%$ inhibition. The initial animal-level prevalence of BLV based on serum ELISA was 20% in animals >6 mo of age, ranging from 9% to 31% at the herd level. Farms were visited quarterly from January 2024 to January 2025, and all animals at risk (i.e., animals that did not have a previous positive BLV test) were sampled at each visit. In total, 245 (5%) of the animals at risk seroconverted over 12 mo, ranging at the farm level from 0% to 16% annual incidence risk of apparent new infection. The average time from first test to seroconversion for animals that contracted BLV was 189 d (± 67 d). In total, 3,707 animals (69%) remained BLV test-negative. There were 1,367 animals (26%) that initially tested negative for BLV but did not receive a follow-up test, usually because they were

culled. Based on these preliminary results, annual new infection risk varies considerably among herds. Further analysis will reveal risk factors associated with transmission of BLV in these herds and contribute to understanding of BLV transmission dynamics.

Key Words: animal health, disease

2331 Enhanced antimicrobial activity of chitosan nanoparticle-encapsulated bovine lactoferricin against mastitis pathogens in dairy cows. K. M. Wang^{*}, J. J. Tong, and H. Zhang, *Beijing University of Agriculture, Beijing, China.*

Bovine mastitis affects milk production, animal welfare, and economic sustainability. Although antibiotics remain essential, concerns about bacterial resistance have driven interest in alternative treatments. This study evaluates the antibacterial efficacy of bovine lactoferricin-loaded chitosan nanoparticles (BLfcin-NPs) against mastitis-causing pathogens as a potential complementary therapy. The BLfcin-NPs were synthesized using ionic gelation with sodium tripolyphosphate (TPP) as a crosslinking agent. Characterization included particle size, polydispersity index (PDI), and zeta potential, measured via dynamic light scattering (DLS) analysis. Encapsulation efficiency was determined using spectrophotometry. Antibacterial activity was evaluated using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays following Clinical and Laboratory Standards Institute guidelines against *Staphylococcus aureus* (ATCC 29740, American Type Culture Collection) and *Escherichia coli* (CVCC 1450, Center for Veterinary Culture Collection). Time-kill kinetics were evaluated over 24 h, with bacterial counts measured at multiple time points via serial dilution and plating. Meanwhile, transcriptomic analysis was performed on these bacteria. Statistical analysis included a 2-tailed *t*-test ($P < 0.05$) to compare antibacterial effects of BLfcin and BLfcin-NPs. The results indicated that BLfcin-NPs exhibited an average size of 101.2–135.9 nm, a zeta potential ranging from +19.83 to +21.37 mV, and an encapsulation efficiency of 16.36%–72.18%. Compared with BLfcin, BLfcin-NPs exhibited significantly enhanced antibacterial activity, with MIC values of 80 $\mu\text{g/mL}$ for *S. aureus* and 320 $\mu\text{g/mL}$ for *E. coli*. Time-kill kinetics confirmed sustained antibacterial effects for up to 24 h. Transcriptomics analysis identified differential gene expression related to ribosomal function, ABC transporters, and 2-component regulatory systems. The BLfcin-NPs demonstrated superior antibacterial efficacy compared with BLfcin alone, supporting their potential as an adjunct therapy for bovine mastitis. This nanotechnology-based approach may reduce antibiotic use while maintaining milk safety and mitigating bacterial resistance.

Key Words: bovine lactoferricin, *Staphylococcus aureus*, transcriptomics

2332 We'll have milk, hold the *Brucella*: Shedding of *B. abortus* RB51 in mammary gland secretions. E. J. Putz^{*1}, K. Sarlo Davila², P. Boggiatto¹, B. Petry^{1,3}, L. G. V. Fernandes^{1,3}, B. A. Crooker⁴, and S. C. Olsen¹, ¹Infectious Bacterial Diseases Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Ames, IA, ²Ruminant Diseases and Immunology Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Ames, IA, ³Oak Ridge Institute for Science and Education, Oak Ridge, TN, ⁴Department of Animal Science, University of Minnesota, St. Paul, MN.

Brucella abortus is the bacteria agent causing brucellosis, a global zoonotic disease of clinical and veterinary importance. In cattle, *B. abortus* causes reproductive distress including abortions, weak calves, as well as

rebreed complications. The *B. abortus* modified live vaccine RB51 has been successful in controlling and preventing disease, however, within the US, a small selection of dairy cows have recently been shown to shed RB51 in their milk. This observation has thus far been most heavily documented to naturally occur in Jersey cows, suggesting a potential influence of host genetics. At the National Animal Disease Center, we have deeply characterized a Jersey cow chronically shedding RB51 in her mammary gland secretions. To investigate Holstein response to RB51 we used 2 distinct Holstein genotypes representing contemporary dairy genetics as seen in commercial herds, and research animals that have been unselected for milk production and other traits since the early 1960s. Previously, these genotypes were shown to have divergent responses to RB51 in vitro exposure. In this study, we collected blood and milk from contemporary and unselected lactating dairy cows pre- and post-vaccination with RB51. Although one contemporary cow shed RB51 in her milk, differences in peripheral immune cell gene expression were evident between genotypes. This work investigates pathogenesis of bacterial shedding in the mammary gland and highlights potentially underrecognized exposure to vaccine in milk. This study is of interest to dairy producers, veterinarians, and researchers working on *Brucella* and similar bacterial diseases.

Key Words: brucellosis, zoonotic, genetics

2333 Effects of incremental levels of *Ascophyllum nodosum* meal on Jersey cows during a feed restriction. W. M. Sims*, M. A. Rahman, K. V. Almeida, M. A. Arshad, M. M. H. Khandakar, M. G. M. Carvalho, O. Osuolale, L. C. R. Junior, and A. F. Brito, *University of New Hampshire, Durham, NH*.

Phlorotannin, a unique bioactive compound of the brown macroalgae *Ascophyllum nodosum* (ASCO), has shown inhibitory effects on α -amylase activity, which may affect the energy balance of dairy cows during feed restriction (FR). Previous research in our laboratory showed a linear decrease in NEFA when dairy cows were fed incremental amounts of ASCO meal. We aimed to evaluate specific metabolic responses of Jersey cows fed ASCO meal under FR. Six multiparous organic-certified Jersey cows (121 ± 12 DIM) were randomly assigned to 1 of 3 treatments: 0 (control = CON), 170 (medium = MED), and 340 g/d (high = HI) of ASCO meal in a replicated 3×3 Latin square design. Cows were fed a TMR (15.9% CP, 34.1% NDF) with 52% grass-legume baleage and 48% ground corn/soybean meal-based concentrate. Periods lasted 25 d, with 19 d adaptation and 6 d for sample collection. During the 6-d sampling period, the first 3 d were used as baseline, followed by FR throughout the remaining 3 d. A 40% FR was calculated based on the average DMI over 5 d preceding the FR, with 10% of the remaining TMR replaced by a low-quality grass hay (8.5% CP, 58.5% NDF). Data were analyzed using the MIXED procedure of SAS with fixed effects of treatment, time (i.e., day), and their interaction, and random effect of cow within square. Preplanned orthogonal contrast was used to assess linear and quadratic effects. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. Intake of DM was similar prior ($P = 0.41$; mean = 15.0 kg/d) and during ($P = 0.76$; mean = 10.6 kg/d) FR. Diets had no effect ($P \geq 0.37$) on the serum concentrations of NEFA, BHB, and glucose. A day effect ($P < 0.01$) was observed for NEFA, which was the greatest at d 2 of FR (mean = +460 μ Eq/L units) compared with the baseline, and glucose being lower ($P = 0.05$) on d 1 compared with d 2 and 3 of FR. A day effect ($P < 0.01$) was also found for BW (mean = -23.5 kg) and energy-corrected milk yield (mean = -8.76 kg) at d 3 of FR compared with the baseline. Our data showed that

ASCO meal did not change the specific metabolic responses measured on Jersey cows during FR.

Key Words: feed restriction, seaweed, stress

2334 Comparative genomic analysis of clinical *Salmonella* Dublin isolates from dairy cattle in Florida. A. R. DeZoysa*, L. K. Edison², A. J. Pellissery^{2,3}, Y. R. Bommineni³, D. Coiner³, D. Simon³, T. Denagamage¹, and S. Kariyawasam², ¹Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, ²Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida, Gainesville, FL, ³Bronson Animal Disease Diagnostic Laboratory, Kissimmee, FL.

The objective was to evaluate the genomic similarities and differences among clinical isolates of *Salmonella* Dublin obtained from the Bronson Animal Disease Diagnostic Laboratory (Kissimmee, FL). There were 7 *Salmonella* Dublin isolates in the repository, all isolated from cattle in Florida. The whole genome sequencing was performed for the 7 *Salmonella* Dublin isolates using Illumina and Oxford Nanopore technologies. Comparative genomic analysis was conducted using the CLC Genomics Workbench v.25.0.1, Rapid Annotation using Subsystems Technology, Comprehensive Antibiotic Resistance Database, ResFinder, and Proksee. The average chromosome size of the *Salmonella* Dublin strains was 4,851,538 bp, ranging from 4,844,157 (SAL-21-0023) to 4,888,369 bp (SAL-20-0015). The number of plasmids among the isolates varied from 1 to 5, with plasmid sizes ranging from 30,166 (SAL-21-0023) to 159,851 bp (SAL-21-0023). The average GC content of 7 genomes analyzed was 52.11%, ranging from 52.07 (SAL-21-0023) to 52.13% (SAL-19-0013, SAL-19-0015, SAL-20-0033). The average number of coding sequences in the chromosomes was 4,618, where the lowest in SAL-19-0015 (4,606) and the highest in SAL-20-0015 (4,665). Average nucleotide identity (ANI) values of 7 chromosomes ranged from 98.51% to 99.99%, indicating a high level of genomic similarity with the reference chromosomal genome (CP117367) downloaded from NCBI. Plasmids of these isolates harbored multiple antimicrobial resistance genes, including *aph* (3'')-Ib, *aph*(6)-Id, *bla*CMY-2, *floR*, *sul2*, and *tet*(A). The majority of these isolates exhibited resistance to aminoglycosides, β -lactams, phenicols, sulfonamides, and tetracyclines, indicating multidrug resistance. Virulence factor analysis identified the *spv* operon (*spvRABCD*) on virulence plasmids in all 7 strains, which plays a crucial role in severe enteritis and systemic infection in cattle. The *gogB* gene, which encodes an anti-inflammatory effector protein that limits tissue damage during *Salmonella* infection, was found in only one (SAL-20-0015) of the 7 tested isolates. These findings provide valuable insights for future studies aimed at developing effective intervention strategies to mitigate the impact of *Salmonella* Dublin infection in cattle.

Key Words: comparative genomics, *Salmonella* Dublin, dairy cattle

2335 The effect of pulsed electromagnetic field therapy on circulating inflammatory markers in organic dairy cows. F. C. Pharo*, K. A. Young, and C. R. Seely, *University of New Hampshire, Durham, NH*.

Pulsed electromagnetic field therapy (PEMF), a form of electromagnetic stimulation, is used as an adjuvant therapy to aid the body's healing processes. Although most PEMF studies focus on the health effects in humans and companion animals, our research aims to explore the effect

of PEMF on circulating inflammatory markers in healthy lactating dairy cows. Mid-lactation, organic Jersey cows ($n = 36$) of various parities were randomly assigned to 1 of 3 treatments (TRT); 1) control (CON; $n = 12$); no PEMF therapy, 2) once daily PEMF (PEMF1; $n = 12$), or 3) twice daily PEMF (PEMF2; $n = 12$). Treatment consisted of 8 min of PEMF on the topline, followed by 4 min of PEMF on each udder quarter, and was administered immediately after the AM (PEMF1 and PEMF2) and PM (PEMF2) milkings. Cows were enrolled on the study for 10 consecutive days; 2 d of baseline (BL) where no treatment was given, 3 d of treatment (TX), and 5 d of follow-up (FU). Composite milk samples were collected twice daily during all study periods and blood samples were collected at d 1 of BL, d 2 and 3 of TX, and d 2 of FU. Serum was analyzed for albumin (ALB) and serum amyloid A (SAA). Only 25% of samples exceeded the reference threshold for SAA (>5 g/dL), thus was not statistically analyzed. Mixed linear models were created to assess the difference in ALB between TRT groups for BL, TX, and FU periods. Study day, when appropriate, and TRT were retained in all models regardless of significance. For all study periods, ALB was similar between TRT groups (all $P > 0.1$). Parity differences existed for all study periods (all $P < 0.05$), where primiparous cows (BL: 3.94 [95% CI: $3.87, 4.82$] g/dL; TX: 3.96 [3.89, 4.04] g/dL, FU: 3.91 [3.84, 3.99] g/dL) had greater ALB concentrations than multiparous cows (BL: 3.79 [3.76, 3.86] g/dL; TX: 3.79 [3.72, 3.87] g/dL; FU: 3.77 [3.69, 3.84] g/dL). Although we saw no effects of PEMF on inflammation, further investigations of cows with inflammation are needed.

Key Words: pulsed electromagnetic field therapy, inflammation, organic dairy

2336 Early lactation health and milking behavior in cows under an automatic milking system. C. Hernandez-Gotelli¹, D. Manriquez², and P. Pinedo^{*1}, ¹Colorado State University, Fort Collins, CO, ²AgNext, Colorado State University, Fort Collins, CO.

Automatic milking systems (AMS) collect abundant cow-level information that could be used to detect behavioral changes that identify sick cows. The objective was to examine the associations of milking behavior and performance variables with early lactation diseases in cows using an AMS. This study was conducted on a commercial dairy operation in Colorado. The data included 5,222 lactations of multiparous Holstein cows that calved between January 2023 and April 2024 and were milked using 62 AMS. Milking behavior and performance data included milk yield (kg), milking duration (min), milking interval (h), milk flow duration (sec), mean milk flow (kg/min), and the frequency of teat not found, milking unit kick-off, and incomplete milking events for every AMS visit. Health disorders from 0 to 21 DIM, included clinical hypocalcemia (HCa), metritis, mastitis, and displaced abomasum (DA). A healthy group was used as a reference for comparisons. Milking behavior and performance were examined from 1 to 21 DIM for sick and healthy cows. Multivariable mixed linear and logistic regression models for repeated measures were fit using milking behavior and performance variables as the responses of interest. Lactation number and milk yield (when appropriate) were included as fixed covariates, and cow ID was considered a random effect. Compared with healthy cows, milk yield was lower in cows with HCa, metritis, mastitis, and DA. Cows that developed metritis, mastitis, and DA had longer milking durations than healthy cows. Longer milking intervals were identified for all the diseases in analysis. Cows affected by metritis, mastitis, and DA had longer milk flow duration, whereas cows with metritis and mastitis had lower mean milk flow compared with healthy controls. The probabilities of incomplete milking and teat not found were greater only for mastitic cows. As anticipated, health disorders associated with pain

or discomfort significantly affected milking behavior and performance during early lactation. In conclusion, differences in milking behavior and performance between sick and healthy cows were established for most of the disorders in analysis. Integrating changes across various milking variables can enhance the early detection of diseases in AMS.

Key Words: milking behavior, robotic milking, health

2337 Impact of heat stress on intramammary *Escherichia coli* challenge in lactating Girolando cows: Effects on body temperature, plasma insulin, cortisol, and milk yield. A. S. Silva^{*1}, J. Diavão¹, L. C. Mendonça¹, M. M. Campos¹, W. A. Carvalho¹, and J. A. Negrão², ¹Empresa Brasileira de Pesquisa Agropecuária - EMBRAPA Gado de leite, Juiz de Fora, Minas Gerais, Brazil, ²Faculdade de Zootecnia e Engenharia de Alimentos (FZEA), Universidade de São Paulo (USP), Pirassununga, São Paulo, Brazil.

This study aimed to evaluate the effects of lipopolysaccharide of *Escherichia coli* (LPS) challenge under heat-stressed cows on vaginal (VT; iButton, Whitewater, WI), eye, and udder thermal temperatures (T420; FLIR Systems Inc., Wilsonville, OR), plasma insulin, cortisol and milk yield. Twenty-four Girolando cows (3/4 Holstein $\times$ 1/4 Gir, with 111 ± 54 DIM, and 19.4 ± 4.5 kg/d of milk yield) were randomly assigned in a block design with 2 treatments: control (CT) and heat stress (HS). The CT cows remained under a cooling system in freestall (temperature-humidity index [THI] of 72) and HS cows were submitted to a THI of 84 in a climatic chamber from 0700 to 1600 h, for 13 d. All cows were submitted on the 10th day to a single dose of LPS challenge in the left rear udder (100 μ g of LPS diluted into 10 mL of saline solution 0.9% NaCl) and received 10 mL of saline solution (0.9% NaCl) in the right rear udder. The VT was measured every hour during the LPS challenge (from 10 to 13 d); blood samples were collected at -24, 0, 4, 6, 10, 24, 48, 72 h relative to the LPS challenge; and eye and udder temperatures were measured at 0, 1, 4, 6, 24, 48, and 72 h of LPS challenge. Data were analyzed using a complete block design (SAS Institute Inc.) with repeated measures. Significance was considered when $P \leq 0.05$. The HS cows had greater VT during the LPS (HS = 39.1 vs. CT = 38.7°C ; $P = 0.01$). Heat-stressed cows had greater eye (HS = 39.1 vs. CT = 38.3°C), right rear udder (HS = 38.7 vs. CT = 37.7°C), and left rear udder (HS = 38.7 vs. CT = 37.9°C), temperatures at 4 h ($P < 0.05$). Cortisol was greater only at 12 h after the LPS in HS cows (HS = 107.8 vs. CT = 86.2 ng/mL; $P = 0.01$); insulin increased in HS cows only at 12 h after the LPS (HS = 1.87 vs. CT = 1.09 ng/mL) and 24 h after the LPS (HS = 2.10 vs. CT = 1.21 ng/mL; $P = 0.04$). Milk yield was not influenced by heat stress during LPS challenge (HS = 15.0 vs. CT = 15.6 kg/d; $P = 0.24$). Although HS cows exhibited greater body temperatures during the LPS challenge, along with increased cortisol and insulin, HS cows did not decrease milk yield during LPS challenge.

Key Words: cortisol, immune response, tropical climate

2338 Effect of intra-mammary infusion of a carvacrol-based botanical product at dry-off on udder health. P. Munoz-Boettcher^{*1}, C. Hernandez-Gotelli¹, C. Ibarguren¹, R. W. Zheng¹, J. Velez², A. De Vries³, E. K. Miller-Cushon³, B. J. Heins⁴, R. A. Lynch⁵, Q. Kolar⁵, G. M. Schuenemann⁶, V. E. Cabrera⁷, E. Silva⁷, and P. Pinedo¹, ¹Colorado State University, Fort Collins, CO, ²Aurora Organic Farms, Platteville, CO, ³University of Florida, Gainesville, FL, ⁴University of Minnesota, Morris, MN, ⁵Cornell University, Ithaca, NY, ⁶The Ohio State University, Columbus, OH, ⁷University of Wisconsin, Madison, WI.

Table 1 (Abstr. 2338). Distribution (%) of the main bacteriological isolates found at dry-off and during the first (W1) and second (W2) week postpartum by dry-off treatment¹

Item	Dry-off (n = 203)		W1 (n = 155)		W2 (n = 151)	
	CON	BT	CON	BT	CON	BT
No growth	40.0	46.2	54.5	59.3	48.6	60.5
<i>Staphylococcus aureus</i>	20.0	17.3	18.2	19.8	23.0	27.6
Non- <i>aureus</i> staphylococci	34.3	28.8	22.1	12.3	21.6	2.6
<i>Escherichia coli</i>	2.9	1.0	1.3	1.2	0.0	0.0
Other	1.9	3.8	3.9	4.9	4.1	9.2
Contaminated	1.0	2.9	0.0	2.5	2.7	0.0

¹No statistically significant differences between CON and BT were identified.

The objective was to evaluate the effect of intra-mammary infusion of a carvacrol-based botanical product, formulated to maintain or restore the normal uterine environment, on udder health in the subsequent lactation of Holstein cows managed in an organic system. A total of 203 cows were enrolled at the time of dry-off and were assigned randomly into 1 of 2 treatment groups: (1) control (CON; n = 101; average parity = 3.37) receiving 10 mL of distilled water in each quarter and (2) botanical therapy (BT; n = 102; average parity = 3.16), receiving 10 mL of an intramammary infusion of Optimum UterFlush (Van Beek Natural Science, Orange City, IA), diluting one syringe of 30 mL in 1,000 mL of distilled water. Composite milk samples for bacteriological culture and SCC were collected at dry-off (DO) and during wk 1 (W1) and wk 2 (W2) postpartum. Outcomes of interest consisted of SCC and bacterial infection at W1 and W2 and clinical mastitis and culling within the first 30 DIM. Data were analyzed using logistic regression by univariable models followed by multivariable models considering parity number, days dry, and bacterial growth and SCC at DO as covariables. Table 1 shows the pathogen distribution by sampling time. No treatment effect on the probability of a SCS <4 (200,000 SCC/mL) on W1 and W2 was observed. There was no treatment effect on the distribution of cultured pathogens at W1 and W2 when no growth versus growth, gram-positive versus gram-negative, and major versus minor pathogen categories were compared. No differences were observed between treatment groups for clinical mastitis or culling within the first 30 DIM. In conclusion, no effects on udder health, for parameters assessed, were identified following an intra-mammary infusion at dry-off of the botanical product analyzed in this trial.

Key Words: mastitis, organic, dry-off

2339 Molecular characterization and resistance modulation strategies against methicillin-resistant *Staphylococcus aureus* from cattle mastitis. M. Ijaz*, M. U. Javed, A. Ahmed, H. Rasheed, and A. A. Jabir, *University of Veterinary And Animal Sciences, Lahore, Punjab, Pakistan.*

The emergence of antimicrobial resistance (AMR) poses a major challenge, reducing the range of therapeutic choices for both veterinary and public health professionals. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important antimicrobial-resistant strains of *S. aureus* for causing subclinical mastitis in cattle around the globe. For this purpose, 384 milk samples from the lactating cattle were collected using convenient sampling techniques. The samples were initially screened for subclinical mastitis (SCM) by using the California mastitis test. The SCM-positive samples proceeded to standard microbiological procedures for the isolation of *Staphylococcus aureus*. The *S. aureus* isolates were confirmed as MRSA based on the cefoxitin disk diffusion test and the presence of the *mecA* gene by PCR followed by

phylogenetic analysis using bioinformatics tools. The prevalence was determined using the formula described by Thrusfield, whereas the categorical data for antimicrobial susceptibility tests were analyzed as percentages through descriptive statistics. The results revealed that out of 384 milk samples, 233/384 (60.68%) samples were positive for subclinical mastitis, whereas the prevalence of *S. aureus* was noted to be 29.42%. The phenotypic and genotypic prevalence of MRSA was noted to be 41.59% and 24.78%, respectively. The findings of the phylogenetic analysis of the *mecA* gene revealed a strong evolutionary connection between the study isolates and MRSA isolates of Pakistani cattle and buffalo of different studies. Antimicrobial susceptibility testing of MRSA isolates exhibited the highest resistance to penicillin, followed by gentamicin and oxytetracycline, whereas moxifloxacin and linezolid were among the susceptible antibiotics. Synergy testing using the checkerboard assay demonstrated a synergistic effect between penicillin and ketoprofen, gentamicin and flunixin meglumine, and oxytetracycline with *N*-acetylcysteine. The current study concluded that MRSA is an emerging and prevailing issue associated with subclinical mastitis in cattle and the use of antibiotics in combination with non-antibiotics as potential therapeutic agents for resistance modulation against MRSA. This study will help to devise treatment and control strategies against cattle mastitis

Key Words: antimicrobial resistance (AMR), methicillin-resistant *Staphylococcus aureus* (MRSA), *mecA*, cattle, phylogenetic analysis

2340 Control of spinose ear tick in dairy cows with permethrin spray. P. Jardon*, A. Rico^{2,3}, A. Gerry⁴, B. Karle⁵, and N. Silva-del-Rio^{2,3}, ¹Iowa State University, College of Veterinary Medicine, Ames, IA, ²UC Davis School of Veterinary Medicine, Population Health and Reproduction, Davis, CA, ³Veterinary Teaching and Research Center, Tulare, CA, ⁴University of California Riverside, Entomology, Riverside, CA, ⁵University of California Agriculture and Natural Resources, Davis, CA.

The objective of the present study was to evaluate the effectiveness of a 0.50% permethrin spray (Catron IV, Elanco Animal Health) in reducing spinose ear tick (*Otobius megnini*) infestation in lactating dairy cows and its impact on milk yield. The trial was conducted on a 2,400-cow freestall commercial dairy in California with a known spinose ear tick infestation. The study was implemented from October to November 2024. A total of 647 Holstein cows that met the selection criteria (90–180 DIM, ≥35 kg daily milk yield, and ≤500,000 somatic cell count) were enrolled. Eligible cows were housed in 2 different pens and randomly allocated within pen to either control (CON; no treatment, n = 300) or tick treatment (TT; n = 347) group based on the odd/even number of their ear tag ID. Cows assigned to treatment group received ≈3 mL application of 0.5% permethrin spray inside each ear at study initiation and again 15 d later. Researchers evaluated tick infestation weekly using a 4-point tick score (TSc: 0 = no ticks, TSc: 1 = 1–5 ticks, TSc: 2 = 6–10 ticks, TSc: 3 = >10 ticks); both ears were assessed, and the highest score from either ear was recorded as the score for that animal. Daily milk yield data were collected for 4 weeks, and changes in weekly milk yield averages were analyzed. An ordinal logistic mixed model was used to assess treatment effects on TSc, whereas a linear mixed model evaluated treatment effects in weekly milk yield. At enrollment, no significant differences were observed between groups in parity ($P = 0.97$), weekly milk yield ($P = 0.15$), DIM ($P = 0.12$), or tick infestation scores ($P = 0.39$). A significant treatment-by-week interaction ($P < 0.01$) was observed. By wk 3, the distribution of TSc 0, 1, 2, and 3 was 3.6%, 3.2%, 11.7%, and 81.4% in CON vs. 80.5%, 18.0%, 1.5%, and 0% in TT, respectively. However, no significant difference was observed

with treatment or treatment-by-week interaction on milk yield, with a mean milk yield of 39.6 kg/d (TT) and 39.7 kg/d (CON). Our results suggest that 0.50% permethrin spray effectively reduces spinose ear tick infestation in lactating dairy cows but does not significantly affect milk yield over 4 weeks.

Key Words: dairy cow, spinosa ear tick, permethrin

2341 Molecular typing and antimicrobial resistance patterns of *Moraxella bovis* associated with bovine pinkeye in Pakistani dairy herds. M. Avais*¹, H. Mubeen¹, J. A. Khan¹, and I. Rashid², ¹*Department of Veterinary Medicine, University of Veterinary and Animal Sciences, Lahore, Pakistan*, ²*Department of Parasitology, University of Veterinary and Animal Sciences, Lahore, Pakistan*.

Infectious bovine keratoconjunctivitis (pinkeye) is a highly contagious ocular disease in cattle, caused by *Moraxella bovis*, and poses a significant economic burden on dairy farming. This study aimed to molecularly identify *M. bovis* and evaluate its antimicrobial susceptibility from pinkeye-affected dairy cattle. Lacrimal secretions were

collected from 145 clinically affected dairy cows and cultured under aerobic conditions. Of these, 111 isolates were identified as *M. bovis*, and 33 were further subjected to PCR for molecular characterization. Additionally, in vitro antimicrobial susceptibility testing was performed using the disk diffusion method, followed by in vivo efficacy trials of the most effective antibiotics in cattle. Results showed that 76% of the samples were positive for *M. bovis* on culture media. The PCR analysis confirmed the presence of *M. bovis* in 25 isolates, showing a 1,541 bp amplicon on gel electrophoresis. Phylogenetic analysis of the 16S rDNA sequences from 3 isolates demonstrated genetic homology with strains from Uruguay and the United States. Antimicrobial susceptibility testing revealed that *M. bovis* isolates were most sensitive to tobramycin, followed by oxytetracycline and enrofloxacin. The findings from in vitro susceptibility testing were consistent with the results of in vivo clinical trials. In conclusion, *M. bovis* is the primary etiological agent of bovine pinkeye in Pakistani dairy herds, and tobramycin, oxytetracycline, and enrofloxacin are the most effective antibiotics for treatment.

Key Words: pinkeye, antibiotics, dairy cow

Breeding and Genetics 2: Posters

2342 Assessment of inbreeding depression on stayability of Italian Holstein cows. J. C. C. Panetto^{*1,5}, C. Maltecca², A. Fabris⁴, M. Ablondi³, J.-T. van Kaam⁴, R. Finocchiario⁴, S. Callegaro¹, M. Cas-sandro⁴, C. Cipolat-Gotet³, A. Zanotti³, and F. Tiezzi¹, ¹*Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, Florence, Italy*, ²*Department of Animal Science, North Carolina State University, Raleigh, NC*, ³*Department of Veterinary Science, University of Parma, Parma, Italy*, ⁴*Associazione Nazionale Allevatori della Razza Frisona, Bruna e Jersey Italiana (ANAFIBJ), Cremona, Italy*, ⁵*Embrapa Dairy Cattle, Juiz de Fora, Brazil*.

Stayability, expressed as a categorical trait, defines the ability of females to survive from one period to the next. We studied the impact of inbreeding on the stayability of Italian Holstein cows across 3 distinct intervals following their first calving: stay12, representing survival to the second calving; stay23, indicating survival to the third calving; and stay34, for survival to the fourth calving. Data were sourced from the Italian Holstein, Brown Swiss, and Jersey Breeders Association (ANAFBJ) in Cremona, Italy. Sample sizes for cows with valid records and genotypes ranged from 12,000 to 48,000. Individual inbreeding coefficients (ped_Fi) were derived from a pedigree file containing 212,000 records. Genomic inbreeding was estimated via 3 methods: Fgrm, derived from the diagonal of the genomic relationship matrix; Froh_genome, calculated as the ratio of the sum of runs of homozygosity (ROH) segments to the entire genome; and Froh_class, which categorizes ROH segments into 6 classes based on their sizes. Variance components and fixed effects were estimated using a Bayesian framework with the software *thrgibbs1f90*. Inbreeding was set as a covariate, and slopes were first estimated on the liability scale then converted to the probability scale. Models using genomic measures better captured the effects of inbreeding, as compared with ped_Fi. Among genomic metrics, no significant differences were observed. Notably, the Froh_class analysis indicated that inbreeding depression was significant only for ROH segments of intermediate and larger sizes. Using Froh_genome on the pedigree scale, inbreeding depression slopes were -0.42 for stay12, -1.16 for stay23, and -0.59 for stay34, representing the expected changes in the probabilities of success for each 1% increase in inbreeding. Thus, the expected decays from the least to the most inbred cows were 12%, 31%, and 16%, respectively. Inbreeding resulted in significant reductions in the stayability of Italian Holstein cows. Implementing strategies that employ genomic predictions of inbreeding could serve as a valuable tool to mitigate these detrimental effects and ensure resilience in this population.

Key Words: dairy cattle, runs of homozygosity (ROH), survival

2343 Application of generation proxy selection mapping to detect polygenic loci under selection in Holstein cattle. H. Mulim^{*1}, G. Campos Soares², J. P. Boerman¹, and H. Rojas de Oliveira¹, ¹*Purdue University, West Lafayette, IN*, ²*Interbull Centre, Uppsala, Sweden*.

Generation proxy selection mapping (GPSM) is a powerful approach for detecting genetic loci under selection by tracking allele frequency changes over time. In this study, we applied GPSM to identify genomic regions in Holstein cattle that exhibited significant allele frequency shifts. Genotypic data from 14,469 Holstein cattle born between 2011 and 2019 were analyzed. Animals were genotyped using different SNP panels (ranging from 13K to 70K) and imputed to a common marker density of 68,029 SNPs. Quality control procedures were performed in

PLINK, filtering out markers with low call rates (<0.90), extreme deviations from Hardy-Weinberg equilibrium ($P < 10^{-5}$), and minor allele frequency <0.01 , resulting in 56,025 SNPs for analysis. The GPSM was performed using a genome-wide linear mixed model implemented in GCTA, with birth year as the dependent variable. The Bonferroni multiple-test correction was used to determine the significance threshold ($\alpha = 0.05/\text{number of SNPs}$). A total of 27 significant SNPs located on 12 chromosomes exhibited significant allele frequency changes over time. These SNPs were located near 92 annotated genes, including 58 protein-coding genes, 16 long non-coding RNAs, 1 pseudogene, 1 microRNA, 11 small nucleolar RNAs, and 4 small nuclear RNAs. Functional analysis revealed enrichment in 32 gene ontology terms, including the Estrogen signaling pathway (KEGG: 04915) and *Staphylococcus aureus* infection (KEGG: 05150). Additionally, 19 quantitative trait loci were associated with key traits related to health (e.g., bovine viral diarrhea virus susceptibility, bovine respiratory disease susceptibility, hematocrit levels, tick resistance, immunoglobulin G level), body conformation (stature), milk production (e.g., milk yield, fat and protein content, riboflavin concentration, milk myristic acid content), and reproduction (abnormal sperm percentage). This study identified genomic regions under selection in Holstein cattle, emphasizing key loci linked to economically important traits under genetic selection over time.

Key Words: allele frequency, genomic selection, selection signature

2344 The impact of service sire breed on age-adjusted production during gestation and subsequent lactation. B. L. Basiel^{*}, A. M. Miles, and P. M. VanRaden, *Animal Genomics and Improvement Laboratory, Agricultural Research Service, United States Department of Agriculture, Beltsville, MD*.

The objective of this study was to determine if service sire breed affects milk and component yield during gestation and in the lactation following calving. Breeding records that resulted in calvings from the years 2017 to 2024 were extracted from the National Cooperator Database. Cow breeds were categorized as Holstein (HO), Jersey (JE), crossbred (XD), and other dairy breeds (OD). Calvings to dairy sire breeds included HO ($n = 7,644,731$), JE ($n = 1,034,937$), and OD ($n = 225,941$) and beef sire breeds included Angus (AN; $n = 1,834,945$), Charolais (CH; $n = 191,366$), Limousin (LM; $n = 232,349$), Simmental (SM; $n = 354,956$), Wagyu (KB; $n = 131,637$), crossbred beef (XB; $n = 297,319$), and other beef breeds (OB; $n = 18,461$). Phenotypes of 305-d age-adjusted milk, fat, and protein yield during the lactation of pregnancy ($n = 11,966,643$) and the lactation of calving ($n = 7,884,390$) were evaluated with generalized linear models where herd-year-season of calving was absorbed. Model effects included sire breed, cow breed, and their interaction, parity (1, 2, 3, 4, and ≥ 5) and cow's PTA of the evaluated trait. Milk yield of cows carrying HO-sired calves was 6 to 91 kg greater than cows carrying AN, LM, SM, CH, JE, and OB-sired calves. Conversely, cows carrying KB and OD-sired calves produced 20 and 34 kg more milk, respectively, whereas those carrying XB-sired calves did not statistically differ in milk yield from cows carrying HO-sired calves. Cows carrying JE and OB-sired calves produced 1.2 and 2.2 kg less fat and 1.0 and 1.7 kg less protein than those carrying HO-sired calves. Fat yield of cows carrying CH-sired calves did not significantly differ from those carrying HO calves but produced 1.1 kg less protein, whereas cows carrying calves sired by other breeds produced 1.2 to 4.4 kg more fat and 0.4 to 3.6 kg more protein. In the next lactation, cows that carried CH-sired calves produced 62, 1.9, and 1.9 kg less milk, fat, and protein, respectively,

than those that had HO-sired calves. Milk and components were similar or greater in cows that had calves sired by other breeds. Sire breed of the calf carried had significant, but mostly small, effects on lactation yields during gestation and in the following lactation, indicating that calf value is more important than effects on the dam.

Key Words: beef × dairy, beef on dairy

2345 Milk fatty acids of Holstein cows compared with Holstein, Montbéliarde, and Viking Red crossbred cows in 2 dairy herds. K. M. Bosley*, L. B. Hansen, and B. J. Heins, *University of Minnesota, St. Paul, MN.*

The health attributes of milk fat have been increasingly scrutinized as consumer interest in functional foods with perceived health benefits continues to grow. The objective of the study was to compare fatty acids in milk of Holstein (n = 424) cows and crossbred cows composed of Montbéliarde, Viking Red, and Holstein breeds (n = 138) from 2 commercial dairy herds in Minnesota. Total, de novo, mixed and preformed FA concentrations, as well as specific fatty acids from test days were provided by Minnesota DHIA (Buffalo, MN) from July 2022 to January 2024. Test-day observations from milk recording were used to fit lactation curves of cows. Lactation curves from Legendre polynomial random regression were compared for 305-d total, de novo, mixed, and preformed fatty acid concentrations, as well as specific fatty acids from the test-day observations of fatty acids. First-lactation and second and greater lactation cows were analyzed separately for herds. Three test days were required per cow. For statistical analysis, fixed effects were breed group, parity (primiparous or multiparous), herd, calving season nested within herd, season, and the interaction of parity and breed group, herd and season. Random effects were cow nested within breed group and herd and DIM. Herd A had higher ($P < 0.05$) preformed and 18:0, 18:1cis-9 FAs than herd B. However, herd B had higher ($P < 0.05$) total, de novo, mixed, and 16:0 FAs. Crossbred cows had higher ($P < 0.05$) de novo fatty acids compared with Holstein cows. The multiparous crossbred cows had higher ($P < 0.05$) de novo fatty acids (27.6 vs. 26.9 g/100 g total FA) and lower ($P < 0.05$) mixed (37.6 vs. 38.3 g/100 g total FA) fatty acids than multiparous Holstein cows. Crossbred cows may have advantages over Holstein cows for fatty acid profile in high-performance dairy herds.

Key Words: crossbreeding, milk fatty acid, lactation curve

2346 Exploring mRNA isoform signatures of feed efficiency in Jersey cattle under varying dietary regimes. F. Muhammad*, V. Asselstine¹, C. F. Baes¹, F. Miglior^{1,2}, F. S. Schenkel¹, and Á. Cánovas¹, ¹Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, Ontario, N1G2W1, Canada, ²Lactanet Canada, Guelph, Ontario, N1K1E5, Canada.

Feed efficiency (FE) is a critical trait in livestock production, reflecting an animal's ability to convert feed into marketable product. Among the various FE metrics, residual feed intake (RFI; kg DMI/day) is increasingly preferred for its ability to account for variations in maintenance and production requirements. Cows that convert gross energy to production more efficiently or require less maintenance than expected for their body weight, tend to consume less feed, resulting in lower RFI. Cows with low RFI are likely to digest and metabolize nutrients more efficiently, leading to improved overall productivity, provided they maintain physiological fitness. The regulation of FE is influenced by both genetic and environmental factors, with moderate heritability indicating a significant role for diet and management in shaping performance. By

enabling high-throughput transcriptome profiling and gene expression analysis, RNA sequencing (RNA-Seq) has emerged as a powerful tool for uncovering the biological mechanisms underlying FE. This study aimed at identifying mRNA isoforms that are differentially expressed (DE) between low- and high-RFI Jersey cows fed both control (C) and high-concentrate (HC) diets. We analyzed publicly available RNA-Seq data (GSE92398) from the NCBI Gene Expression database, derived from liver biopsies (100 paired-end base pairs) of 10 cows (n = 5 high-RFI, n = 5 low-RFI). Quality control and read alignment using CLC Genomics Workbench (v.20.0.4) yielded an average mapping rate of 91% to the ARS-UCD 1.2.11 bovine reference genome (v.110). Differential expression analysis ($FDR < 0.05$, $|FC| > 2$) identified 66 mRNA isoforms upregulated in low-RFI and 37 in high-RFI animals. Functional analysis revealed enrichment of immune and metabolic processes across both dietary treatments, whereas cell signaling and reproductive processes were consistently expressed independently of RFI. These findings provide valuable insights into the molecular basis of FE and contribute to strategies aimed at enhancing efficiency and reducing environmental impact in dairy cattle.

Key Words: feed efficiency, RNA sequencing (RNA-Seq) functional genomics

2347 Milk BHB heritability estimations and genetic correlations with milk yield in confined and grazing dairies from Chile. M. Cai and P. Melendez*, *City University of Hong Kong, Hong Kong, SAR.*

The objective of this study was to estimate the heritability of milk BHB concentrations throughout lactation in Holstein cows from confined and grazing dairies in Chile and its genetic correlation with milk yield. The study used a large database from an official milk recording dairy cooperative (Cooprinsem, Chile). The study covered lactations between 2019 and 2023 (n = 283,362) from both types of dairy. The milk recording evaluated the cow's milk yield on the day of the farm visit, and a milk sample was collected for BHB and other constituent determinations. The visits were conducted once a month for 10 consecutive months, starting from the first month of lactation (T1–T10). The BHB concentrations in milk were measured using infrared technology (Milkoscan equipment). The method used to estimate heritability values and genetic correlations (GC) was the Haseman–Elston regression. The GC between BHB and milk yield throughout lactation slightly varied between confined and grazing dairies. In confined dairies, GC decreased linearly from 0.5 in T1 to –0.5 in T10, whereas in grazing dairies, GC went from 0.4 in T1 to 0.8 in T2 and T4, then abruptly declined to almost 0 in T5 and approximately –0.4 from T6 to T10. Heritability estimates of BHB in milk were lower in grazing dairies than in confined dairies on all the test days. In grazing dairies, heritability was slightly above 0.2 in T1 and T2 but then dropped to less than 0.15 in T3 and ranged from 0.13 to 0.18 between T4 and T10. For confined dairies, the heritability of BHB in milk was from 0.2 in T1 to 0.32 in T2. It then dropped to 0.26–0.27 in T3 and T4, and then 0.33 in T5, dropping linearly to 0.23 in T7 and T8. Then the heritability was 0.3 in T9 and 0.2 in T10. In conclusion, GC between BHB concentrations and milk production were positive between T1 and T5 and then turned out to be negative during the second half of lactation in both grazing and confined dairies. Heritabilities of BHB in milk were higher in confined than grazing dairies, ranging from 0.2 to 0.33, whereas in grazing dairies, heritabilities ranged between 0.13 and 0.2.

Key Words: BHB, heritability, milk yield

2348 Liver gene expression profiles in Holstein cross calves fed various concentrations of B vitamins. K. N. Brost^{*1}, M. L. Pister¹, N. Unnawong², A. Saito³, J. J. Looor¹, and J. K. Drackley¹, ¹*University of Illinois at Urbana-Champaign, Urbana, IL*, ²*Khon Kaen University, Khon Kaen, Thailand*, ³*The National Federation of Dairy Co-operative Associations (ZEN-RAKU-REN), Tokyo, Japan*.

Our purpose was to evaluate effects of feeding varying concentrations of B vitamins to Holstein-Angus cross calves through 8 wk of age on liver gene transcription. The study was a randomized complete block design with calves blocked by BW and serum total proteins. Milk replacer (MR) with custom blends of B vitamins were fed in a step-up step-down protocol. Treatments were basal MR with no added B vitamins (CON); MR with B vitamins above basal (BP; 4.27 mg/kg of B₁, 3.86 mg/kg of B₃, 0.54 mg/kg of B₆, and 18 µm/kg of B₁₂); and MR with B vitamins above the NASEM (2021) recommendations (NP; 28.8 mg/kg of B₁, 18.6 mg/kg of B₃, 9.17 mg/kg of B₆, and 254 µm/kg of B₁₂). Liver biopsies were performed on 15 calves (3 males, 12 females) under veterinary supervision on d 39 of study. Tissue samples were flash-frozen and stored in a -80°C freezer. Samples were prepared for analysis per real-time quantitative PCR protocol and ran with QuantStudio 7 Flex System. Data were transformed with LOG2 and analyzed with MIXED and UNIVARIATE procedures in SAS. The NP group exhibited lower ($P < 0.05$) expression of *IL-10*, *NFKB*, *SLC19A2*, and a tendency for lower ($P < 0.10$) expression of *BHMT2* relative to CON. Greater *IL-10* may impair pathogen response and delay repair of related tissue damage; *NFKB* regulates genes controlling immune responses that may cause inflammation. Lower *BHMT2* may indicate ample re-methylation of Hcy to Met by other means, such as BHMT. Decreased *SLC19A2* may be explained by other genes encoding thiamine transporters because no signs of deficiency were observed. However, greater ($P < 0.05$) expressions of *CAT* and *SHMT* were observed for NP compared with CON. Increased *CAT* may enhance immune function by limiting damage from free radicals. Greater *SHMT* may improve cell growth and DNA synthesis through its role in the folate cycle and one-carbon metabolism. The BP group did not differ from CON for any target genes. Calves fed B vitamins above current recommendations may benefit from decreased inflammation and enhanced immune function and methionine production. Further research on the impact of thiamine receptors is indicated.

Key Words: calf, B vitamin, gene transcription

2349 Heritability and genetic correlations of methane emissions traits in US Holstein cows. G. Martinez-Boggio^{*1}, K. L. Parker Gaddis², L. M. James³, R. Appuhamy³, J. E. Koltes³, E. Sarmikasoglou⁴, R. J. Tempelman⁴, M. J. VandeHaar⁴, R. L. Baldwin⁵, K. F. Kalscheur⁶, E. A. French⁶, H. C. Mantovani¹, H. M. White¹, K. A. Weigel¹, F. PeñaGaricano¹, ¹*Department of Animal and Dairy Sciences, University of Wisconsin-Madison, Madison, WI*, ²*Council on Dairy Cattle Breeding, Bowie, MD*, ³*Department of Animal Science, Iowa State University, Ames, IA*, ⁴*Department of Animal Science, Michigan State University, East Lansing, MI*, ⁵*Animal Genomics and Improvement Laboratory, ARS USDA, Beltsville, MD*, ⁶*Dairy Forage Research Center, ARS USDA, Madison, WI*.

Enteric methane (CH₄) is a major source of greenhouse gas emissions from dairy farming. In addition, CH₄ represents a loss of 6% to 12% of gross energy intake, which could otherwise be available for milk production. Selective breeding is a promising tool for mitigating enteric CH₄ emissions from dairy cattle. We aimed to assess the variability of

alternative CH₄ traits and estimate their heritability and genetic correlations with feed efficiency. We measured CH₄ production (MeP, in g/d), net energy for lactation (NEL), and body weight (BW) in 1,171 mid-lactating Holstein cows, alongside dry matter intake (DMI) and residual feed intake (RFI) in 1,059 of these cows. These measurements were obtained from 33 studies (7 to 10 weeks; 2023 to 2025) at 6 research and commercial farms. Methane emissions were measured using GreenFeed. We calculated 2 residual methane traits: (1) residual methane intensity (RMI), defined as MeP regressed on NEL and metabolic BW, and (2) residual methane yield (RMY), defined as MeP regressed on DMI. Interestingly, both RMI and RMY traits exhibited large variability: RMI ranged from -147 to 162 g/d, and RMY ranged from -146 to 138 g/d. The heritability of MeP, RMI, and RMY were 0.36 ± 0.07 , 0.24 ± 0.06 , and 0.28 ± 0.08 , respectively. Methane production was moderately correlated with RFI ($r_g = 0.52 \pm 0.28$) and highly correlated with RMI ($r_g = 0.86 \pm 0.05$) and RMY ($r_g = 0.83 \pm 0.07$). In addition, residual methane traits were highly correlated among them ($r_g = 0.97 \pm 0.03$). Our findings suggest a promising genomic evaluation for methane emissions in US dairy cows with CH₄ traits that exhibit considerable variability, moderate heritability, and favorable genetic correlation with each other and feed efficiency.

Key Words: residual methane, feed efficiency, genetic parameter

2350 Developing a rapid monitoring system to detect genetic conditions in dairy cattle. G. Condello^{*1}, F. S. Schenkel¹, J. B. Cole^{2,3}, C. Maltecca³, R. E. Jahnel¹, F. Miglior^{1,4}, and C. F. Baes¹, ¹*Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada*, ²*Council on Dairy Cattle Breeding, Bowie, MD*, ³*Department of Animal Science, North Carolina State University, Raleigh, NC*, ⁴*Lactanet, Guelph, ON, Canada*.

Genomic selection has revolutionized dairy cattle breeding by enhancing the accuracy of genetic evaluations and enabling earlier selection decisions. Although this has accelerated genetic progress, it has also increased the rate of inbreeding per year, heightening the risk of genetic conditions that can lead to significant economic losses for producers. In particular, detrimental recessive haplotypes can lead to embryonic loss, congenital abnormalities, and reduced fertility when in a homozygous state. Despite growing awareness of these risks, Canada currently lacks a rapid monitoring system to efficiently detect and manage the spread of these genetic conditions. This project will develop a comprehensive genomic surveillance framework to identify and mitigate the impact of harmful recessive haplotypes in the Canadian dairy population. Key objectives include simulating genotype inheritance patterns to model the transmission and frequency dynamics of detrimental haplotypes, developing an advanced detection algorithm to determine carrier frequency thresholds that would trigger in depth haplotype analysis, and validating this approach using real-world genotypic and phenotypic data from Canadian dairy herds. In addition, this framework will integrate a centralized database that enables real-time tracking of haplotype frequencies to enhance informed breeding decisions. By equipping breeders with tools to monitor and manage the spread of detrimental haplotypes, this research will contribute to balancing genetic improvement and the genetic risks associated with increased inbreeding rates. Implementing a proactive monitoring system will not only enhance the industry's ability to mitigate genetic risks but also improve animal welfare and ensure the long-term sustainability of the Canadian dairy sector.

Key Words: genomic selection, recessive haplotype, genetic diversity

Dairy Foods: Processing—Posters

2351 Bulk tank holding temperature impacts bacterial growth in raw milk pre-pasteurization. Z. D. Wasserlauf-Pepper*, S. J. Reichler, R. L. Weachock, and N. H. Martin, *Cornell University, Ithaca, NY*.

Bacterial growth in raw bulk tank milk before pasteurization can affect the quality of the finished product due to survival of pasteurization conditions (e.g., gram-positive sporeforming and thermotolerant bacteria) or through production of heat-stable enzymes. The Pasteurized Milk Ordinance (PMO) requires raw milk to be cooled to $<45^{\circ}\text{F}$ ($<7.2^{\circ}\text{C}$) within 6 h of milking to slow the growth of microorganisms present in bulk tank raw milk, but farm cooperatives and processing facilities may require more stringent holding temperatures before pasteurization. This study aimed to evaluate the impact of holding temperature and length of time on the growth of certain microbial populations in bulk tank raw milk. Bulk tank raw milk was collected from 15 dairy farms with varying cooling practices and historical raw milk quality. Samples were transported on ice to the laboratory where each sample was aseptically split into 4 separate sterile containers and held at 2.2°C , 3.3°C , 4.4°C , and 7.2°C for 72 h after collection. Samples were tested every 12 h for aerobic plate count (APC), total gram-negative count (GNC), preliminary incubation count (PIC), and coliform count (CC). We analyzed microbial data using a random effects linear regression model including all test types, and a pairwise comparison of each time-temperature combination indicated that the samples held at 2.2°C and 3.3°C have consistently lower predicted concentrations than 7.2°C for APC, GNC, and PIC at both 60 h and 72 h, but no difference was seen for CC across all comparisons. Further, we observed that GNC outgrowth was higher for milk held at 7.2°C starting at 48 h of storage compared with 2.2°C and 3.3°C . Our results suggest that despite conforming to the PMO requirements for raw milk storage before pasteurization, raw milk held for longer periods of time (i.e., $>36\text{--}48\text{ h}$) at 7.2°C represents a risk to finished product quality due to the growth of relevant groups of bacteria. Additional elucidation of the role of specific microbial populations during raw milk holding will further support development of industry guidelines to ensure dairy product quality throughout the grass to glass continuum.

Key Words: raw milk, cooling

2352 Consumption of materials and resources for the production of various grades of micellar casein concentrate powder using microfiltration process. M. A. Rahman*, S. Chauhan¹, S. Ellekuttige¹, K. Sartell¹, S. Beckman¹, M. E. Uddin², and P. Salunke¹, ¹Department of Dairy and Food Science, South Dakota State University, Brookings, SD, ²Department of Animal Science, University of Connecticut, Storrs, CT.

In the dairy processing industry, membrane filtration processes such as microfiltration (MF) are used to produce micellar casein concentrate (MCC) by physically separating milk components. This study assessed materials (milk) and resources (e.g., electricity, steam, water, cleaning agents) used at different MF stages for MCC production. Three batches of MCC with different ratios of total protein to total solids (TP/TS) were produced from 3 milk lots. A total of 15,875 kg of raw milk was sourced from South Dakota dairy farms during November and December of 2024. After receiving the milk at the dairy plant, the milk was stored in a silo and cream was removed by a centrifugal separator. The skim milk was pasteurized at 78°C for 18 s and cooled to 4°C . From this, 5,278 kg of

skim milk was used to produce MCC through MF. The retentates were concentrated to 62% (MCC62), 74% (MCC74), and 85% (MCC85) TP/TS ratio. At each stage, inputs and outputs, including materials, were recorded. The final MCC was spray-dried to obtain powder which was packaged in plastic bags. Each process was replicated thrice. Statistical analysis was performed using R, including one-way ANOVA and Tukey's honestly significant difference test. Electricity consumed for cream separation did not differ among MCC62, MCC74, and MCC85. Pasteurization, membrane filtration electricity, and drying parameters showed no significant differences among MCCs. Diafiltration (DF) water used to produce MCC74 and MCC85 required additional water relative to the MCC62 treatment. Despite adding DF water, filtration time decreased due to a lower total feed volume because of progressive retentate removal. Water was added after significant permeate removal, reducing available feed. We found that MCC74 had a greater feed volume than MCC85 due to less water addition to MCC85, lowering the total feed amount. Retentate from filtration was significantly lower in MCC85 ($P = 0.023$) as more soluble constituents were removed. Cleaning parameters such as chemicals and sanitizers did not vary among MCCs. Optimizing energy and resource usage in MF is crucial for sustainable dairy processing. This study provides insights into reducing material consumption while maintaining MCC production efficiency.

Key Words: microfiltration, micellar casein concentrate, membrane filtration

2353 High-voltage atmospheric cold plasma for enhancing microbial safety of cheese brines: Inactivation of *Listeria innocua*. D. Manoharan*, Z. Wan¹, and S. Govindasamy-Lucey², ¹University of Wisconsin-Platteville, Platteville, WI, ²Wisconsin Center for Dairy Research, Madison, WI.

Brining is a critical step in cheesemaking, contributing to rind formation, microbial inhibition, and modulation of cheese ripening, texture, taste, and aroma. However, despite the high salt concentration (23%) in brine, pathogens such as *Listeria monocytogenes* can survive, posing significant food safety risks. This highlights the need for novel strategies to improve the microbial safety of cheese brines. This study explores the potential of high-voltage atmospheric cold plasma (HVACP) treatment for the removal of *Listeria innocua*, a nonpathogenic surrogate for *Listeria monocytogenes*, in cheese brines. Brine solutions of varying concentrations (15, 19 and 23%) were treated with HVACP using single and multi-donut electrode configurations at 2 voltage levels (50 and 60 kV) and 3 treatment durations (30, 60, and 90 s) in triplicate. Microbial analyses were conducted immediately after treatment and after 24-h of storage. Analysis of variance along with Tukey's sample comparison test was done using JMP statistical software. Results demonstrated that microbial reduction improved with increasing voltage and exposure time across all brine concentrations, correlating with the generation of reactive species ($P < 0.05$). However, reactive nitrogen species (NO_3^- and NO_2^-) increased from 15% to 19% brine concentration but decreased at 23%, whereas H_2O_2 levels decreased progressively with increasing salt concentration irrespective of the plasma treatment conditions. These results prove the importance of brine concentration on reactive species dissolution. After 24 h of storage, plasma-activated brines (PAB) consistently achieved a 6 log cfu/mL reduction ($P < 0.05$) of *Listeria innocua* across all treatment conditions, irrespective of voltage, exposure time, or electrode configuration. These findings demonstrated the importance of extended storage time to enhance microbial reduction in PAB. The

HVACP treatment of brine solution offers an innovative and promising approach to improve microbial safety during cheese brining and ripening, presenting a promising solution for the dairy industry. Future studies will focus on evaluating the antimicrobial efficacy of PAB on the elimination of pathogens in brined cheeses.

Key Words: plasma, cheese, brine, *Listeria*, reactive species

2354 Evaluating flux performance in ultrafiltration and microfiltration to produce milk protein concentrates (MPC) and micellar casein concentrate (MCC) at 62%, 74%, and 85% protein to total solids ratios. S. Ellekuttige*, S. Chauhan, M. A. Rahman, K. Sartell, S. Beckman, and P. Salunke, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Milk protein concentrates (MPC) and micellar casein concentrate (MCC) are valuable high-protein dairy ingredients produced through membrane filtration technologies with different ratios of total protein to total solids (TP/TS). Manufacturing different grades of MPC and MCC requires removing soluble components and adjusting processing parameters. This study evaluates the processing efficiency of ultrafiltration (UF) and microfiltration (MF) to produce MPC and MCC, respectively, to 62%, 74%, and 85% TP/TS ratios. Raw milk was procured from South Dakota farms, cold separated to produce skim milk, and pasteurized (78°C, 18 s) at the South Dakota State University Dairy Plant. Pasteurized skim milk (10,400 kg) was equally divided into 2 lots, and each lot was used to produce MPC and MCC using UF (10 kDa) and MF (0.2 µm), respectively. The UF was performed at an average transmembrane pressure (TMP) of 264 kPa, whereas MF was carried out at an average TMP of 87 kPa. Both processes were carried out at 11°C and incorporated diafiltration and recirculation steps to concentrate proteins to 85% TP/TS. Permeate and retentate samples were collected for efficiency and quality assessment. Treatments were replicated thrice using lots of different milk each time. A statistical comparison of filtration process parameters in manufacturing MPC and MCC at TP/TS of 62% and 74% showed significant differences, with *P*-values of 0.046 and 0.047, respectively. However, no significant differences were observed at 85% TP/TS concentration in the MPC and MCC manufacturing process. At 85%, UF flux dropped significantly (*P* < 0.05). In contrast, MF maintained a better flux rate at 85% TP/TS during the manufacturing of MCC, suggesting that MF retains the flux rate. In conclusion, UF and MF filtration can effectively produce different grades of MPC and MCC, respectively, with MF demonstrating superior flux retention and

lower pressures at higher protein concentrations than UF. These findings provide insights into optimizing process parameters for MPC and MCC production flux rates with different TP/TS ratios.

Key Words: ultrafiltration, microfiltration, flux rate

2355 Alternative thermal treatments for sanitization of equipment presented in Appendix F of the PMO. G. Botz*, H. M. H. Mohamed, and J. Waite-Cusic, *Oregon State University, Corvallis, OR.*

The grade “A” Pasteurized Milk Ordinance (PMO) sets rules and guidelines to produce grade “A” milk in the United States, ensuring that milk is safe to drink. In the PMO, Appendix F allows for the use of heat as an option for the sanitization of food contact surfaces (FCS). Heat can be applied as either hot water (77°C) or steam (outlet temperature: 94°C) with a contact time of 5 min. This recommendation was incorporated into the PMO in the 1930s, and data no longer exist that support validation of these treatments. Our objective was to evaluate the efficacy of these thermal treatments against thermophilic bacteria (*Enterococcus*, *Microbacterium*, and *Streptococcus* spp.) relevant to dairy processing facilities. Individual strains of each genus (*n* = 4) were cultured in tryptic soy broth (TSB; 30°C, 3 d) and 1 mL was spread on tryptic soy agar (TSA) and incubated (30°C, 3 d) to create a lawn. Lawns were harvested with 0.85% NaCl (5 mL). Equal volumes of individual harvests were mixed to create cocktails. Coupons were inoculated with 50 µL of the cocktail and dried in a biosafety cabinet (~3 h). Inoculated coupons were transferred to test tubes containing 0.85% NaCl (5 mL) in a preheated water bath at 63°C, 77°C, or 94°C. Following treatment time, enumeration was performed by serial dilution in 0.85% saline with spread plating on standard methods agar (SMA; 30°C, 3 d). Vegetative thermophilic bacteria were confirmed to withstand pasteurization (63°C, 30 min) with an average reduction of 0.44 ± 0.15 log cfu/coupon. Appendix F thermal recommendations varied substantially in efficacy with the 77°C, 5 min treatment achieving a 1.24 ± 0.48 -log reduction and the 94°C, 5 min treatment achieving a 6.53 ± 1.05 -log reduction. This data indicates that the 94°C, 5 min recommendation in the PMO is an effective sanitization method for FCS; however, the 77°C, 5 min recommendation provides insufficient reduction of thermophilic bacteria on FCS. Future work will investigate the efficacy of these treatments on relevant bacterial spores.

Key Words: thermal sanitization, Pasteurized Milk Ordinance, dairy safety

Dairy Foods: Cheese—Posters

2356 Study on the effects of low-temperature storage on the quality characteristics, microstructure, and shelf life of Halloumi cheese. B. Q. Liu^{*1}, Y. Su¹, L. Guo^{1,2}, Y. J. Jiang^{1,2}, and X. Y. Yang^{1,2}, ¹Key Laboratory of Dairy Science, Ministry of Education, College of Food Science, Northeast Agricultural University, Harbin, China, ²Key Laboratory of Infant Formula Food, State Administration for Market Regulation, Harbin, China.

Low-temperature storage is essential for cheese preservation. Refrigeration (4°C) effectively delays microbial growth, making it suitable for short-term storage. Freezing (−20°C) extends shelf life by inhibiting microbial growth, beneficial for long-distance transport and cross-regional distribution. However, low-temperature storage may affect Halloumi cheese's cooking properties, causing fat exudation and reduced meltability, which in turn impacts processing performance and sensory quality. This study analyzed the quality, microstructure, and shelf life changes of Halloumi cheese, aiming to provide scientific guidance for optimizing storage methods. The raw milk was pasteurized and cooled to 37°C, followed by fermentation with commercial starter culture for 1 h. Rennet was added, and coagulation occurred for 1.5–2 h. The curd was cut and then pressed at 150 kPa for 2 h. Subsequently, the curd was heated in 95°C hot whey for 60 min until it floated. Finally, the cheese was soaked in 10%–15% brine for 10–15 min. The final samples were stored at 4°C and −20°C for analysis. All data were presented as the mean ± SD, with 3 parallel replicates for each group. Statistical analysis was performed using ANOVA and independent samples *t*-test. The results showed that refrigeration led to more significant moisture loss (16.31%) compared with freezing. With extended storage, both freezing and refrigeration significantly reduced pH, protein, fat, and lactose content, but refrigeration slowed this decline, delaying it by at least 14 d. Color analysis revealed significant decreases in L and b values, indicating that darkening may be associated with changes in protein structure and alterations in light-scattering properties due to moisture loss. Freezing significantly affected the microstructure, disrupting the casein network, causing fat exudation, and resulting in a looser, more porous structure. Texture analysis showed both treatments softened the texture, with no significant difference. Rheological analysis showed that storage modulus (G') and loss modulus (G'') initially increased and then declined over time. The early increase in G' and G'' may be attributed to enhanced protein-protein and protein-fat interactions, which contribute to the formation of a stronger network structure. However, as storage progressed, moisture redistribution and ice crystal formation in frozen samples likely disrupted the casein matrix, significantly reducing G' and G'', thereby decreasing hardness and elasticity. In contrast, refrigerated samples exhibited a more moderate change, preserving the protein network and structural stability. Additionally, coliform counts exceeded 100 cfu/g in the frozen and refrigerated samples at 56 and 35 d. In conclusion, although freezing significantly extends shelf life, it damages the microstructure and texture, affecting quality. Refrigeration better preserves nutritional components, making it more suitable for medium to short-term storage.

Key Words: Halloumi cheese, low-temperature storage, quality

2357 Study on the microscopic characteristics, physicochemical properties, and processing feasibility of unheated Halloumi cheese under low-temperature storage. J. Y. Wang^{*1}, C. Yang¹, B. Q. Liu¹, N. Xu¹, L. Guo^{1,2}, and Y. J. Jiang^{1,2}, ¹Key Laboratory of Dairy Science, Ministry of Education, College of Food Science, Northeast

Agricultural University, Harbin, China, ²Key Laboratory of Infant Formula Food, State Administration for Market Regulation, Harbin, China.

In the industrial production of Halloumi cheese, the scalding process is crucial for ensuring its unique texture and heat-resistance properties. However, this process is relatively fixed, limiting the flexibility to quickly adjust product types or quantities based on market demand. Raw milk was pasteurized at 75°C for 3 min, cooled to 33°C, and fermented with *Lactobacillus* at 33°C for 1 h to reach pH 6.8. After 1.5–2 h of rennet curdling, the curds were cut, stirred at 37°C for 15 min, and at 43°C for another 15 min. The whey was drained, and the curd was pressed at 170 kPa for 1.5 h, then cut into 10 cm × 7 cm pieces, demolded, and heated in 90°C whey for 60 min until floating. The cheese was then soaked in brine. Therefore, this study compared fresh Halloumi cheese with that of low-temperature stored, unheated cheese, analyzing the physicochemical properties, textural characteristics, rheological properties, and the feasibility of producing Halloumi cheese products after further processing. Results showed that unheated Halloumi cheese can be processed into a final product within 4 d under freezing and 6 d under refrigeration without significant quality deterioration. However, when the samples exceed the storage period and are subjected to scalding, the resulting cheese gradually softens and begins to melt, making it impossible to achieve Halloumi cheese. Specifically, the reprocessed samples exhibited good stability, with no significant changes in water activity or pH. However, the water content of frozen and refrigerated samples increased significantly by 8.7% and 7.2%, respectively. The hardness of the cheese significantly decreased, particularly under freezing conditions, with a more fragmented texture and poor cohesion. Rheological properties show that with prolonged storage, both storage modulus (G') and loss modulus (G'') decrease, indicating a reduction in elasticity and viscosity. This is due to the degradation or rearrangement of casein and fat during long-term storage, leading to melting during heating. Color analysis revealed a significant decrease in *L** value and an increase in *b**, indicating a darker and more yellowish hue, likely due to structural changes during freezing and refrigeration, along with chemical reactions (Maillard reaction) during scalding. This study reveals the processing potential of unheated Halloumi cheese under freezing and refrigeration, providing a new theoretical basis for its feasibility in these conditions.

Key Words: Halloumi cheese, scalding, texture, storage characteristic

2358 Impact of nisin-protective culture and ripening temperature on histamine production in Gouda cheese. J. J. Larsen^{*1}, R. A. Ibáñez², J. M. Pérez², J. J. Jaeggi², and J. A. Lucey^{1,2}, ¹University of Wisconsin–Madison, Madison, WI, ²Center for Dairy Research, Madison, WI.

Histamine (Him) can be formed during cheese ripening due to decarboxylation of free histidine (FHIs) by bacteria containing histidine decarboxylase enzymes (HDC). Excessive accumulation of Him in cheese (>200 mg/kg) may cause off-flavors, undesirable gas formation, and allergic-like reactions in sensitive consumers. Levels of Him in cheese can be reduced by controlling ripening temperatures (<10°C). Nisin, an antibacterial peptide produced by some strains of *Lactococcus lactis*, has been effectively used in cheese to prevent the occurrence and growth of undesirable bacteria. This study aimed to determine the impact of a nisin-producing *L. lactis* culture and different ripening temperatures on the accumulation of Him in Gouda cheese. Gouda cheeses were manu-

factured with/without a commercial *L. lactis* nisin-producing culture and with/without contamination by *Lentilactobacillus parabuchneri* (a common Him producer) at an inoculation level of 10^1 cfu/mL in the milk ($n = 4$ replicate trials). Cheeses were then ripened at 4 or 10°C and evaluated for composition, pH, levels of Him, non-starter lactic acid bacteria (NSLAB), and counts of DNA sequences corresponding to HDC. All cheese treatments resulted in similar pH values 1 d after manufacture and composition at 14 d ($P > 0.05$), which were typical for Gouda cheese. Preliminary findings after 30 d of ripening showed no significant differences ($P > 0.05$) in levels of Him (<21 mg/kg) and NSLAB ($<10^4$ cfu/g) among treatments; however, the use of a nisin-producing culture in combination with ripening at 4°C significantly reduced ($P < 0.05$) the number of DNA sequences corresponding to HDC in treatments contaminated with *L. parabuchneri* (<100 versus $>1,000$ copies/g). These results suggest the combination of nisin-producing cultures and reduced ripening temperatures can decrease the occurrence of Him producing bacteria in cheese, although assessments at longer ripening times will be required to determine the impact on Him accumulation.

Key Words: *Lentilactobacillus parabuchneri*, cheese microbiology, cheese quality

2359 Impact of lactose control techniques on properties of block rindless Swiss cheese. E. M. Levinson^{*1}, S. Govindasamy-Lucey², and J. A. Lucey^{1,2}, ¹*Department of Food Science, University of Wisconsin–Madison, Madison, WI*, ²*Center for Dairy Research, University of Wisconsin–Madison, Madison, WI*.

Swiss cheese is popular in school lunch programs due to its high protein, low fat, and low sodium contents. However, quality issues have recently been seen with the flavor and texture of block rindless Swiss cheese, including more cheese with splits or blind cheese. Swiss cheese is traditionally made using whey dilution (WD) to control the lactose content, but this may be less effective with the increased use by industry of ultrafiltered (UF) concentrated milk. We studied how different methods for lactose control affected the properties of block rindless Swiss cheese. Three trials ($n = 3$) were completed with 4 Swiss treatments: control cheese (CC) used milk with 2.5% CN and WD (30%) and the other 3 treatments used UF concentrated milk (4.05% CN) and explored water addition to milk (WM), WD, and lactose standardization (LS, to 3.35% lactose) of milk. All concentrated cheese milks were pre-acidified to pH 6.4. Cheese was ripened in a cold room ($\sim 5^\circ\text{C}$) for 10 d, a warm room ($\sim 23^\circ\text{C}$) for 3 wk, then returned to a cold room to continue ripening. The composition of cheese out of the warm room (4 wk) was similar ($P < 0.05$) for all cheeses: $\sim 38\%$ moisture, $\sim 30\%$ fat, $\sim 0.5\%$ salt, and $\sim 27\%$ protein. At 2 mo, the cheese was judged by a licensed Swiss cheese grader: the CC and WD cheeses were given A (top) grades; whereas WM and LS treatments were given D (low) grades. Over 4 mo of ripening, the pH of all cheeses increased and was in the range pH 5.51 to 5.64; the WD cheese had the lowest pH while the CC had the highest pH (and highest lactic acid levels at 10 d). At 4 mo of storage, cheeses were analyzed by a trained sensory panel. Firmness was similar ($P < 0.05$) for all samples. The cheese made with WM had the lowest scores for nutty, buttery, milky, and umami; and scored high on astringent, acid, and sweaty. Desirable eye development (shape, size, and number) was seen for CC and WD cheeses, which used WD. Currently, we are analyzing the detailed impact of WD on cheese composition and microbiology, and comparing this to the 2 other treatments, to optimize the lactose control process. High-quality block rindless Swiss cheese was successfully made from concentrated milk.

Key Words: lactose standardization, block rindless

2360 Characterization of taste by trained panel of Manchego cheese made from raw or pasteurized milk. L. Jiménez Sobrino^{*1}, J. E. Williams¹, I. Castro Navarro², M. K. McGuire², R. Arias Sánchez³, and M. A. McGuire¹, ¹*Department of Animal, Veterinary, and Food Sciences, University of Idaho, Moscow, ID*, ²*Margaret Ritchie School of Family and Consumer Sciences, University of Idaho, Moscow, ID*, ³*Centro de Selección y Reproducción Animal (CERSYRA) – Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha (IRIAF-JCCM), Valdepeñas, Ciudad Real, Spain*.

Cheese is an important sector of the dairy industry worldwide, with global sales totaling \$122.1 billion, as well as in the United States, where the value of cheese surpassed \$74 billion in 2023. Traditional Manchego cheese is an iconic product of Spain made with either raw or pasteurized sheep milk. The objective of our study was to characterize the differences (if any) in taste descriptors in Manchego made from raw or pasteurized milk. A total of 31 imported cheeses (15 raw milk and 16 pasteurized milk) with 60 d of aging were presented to 4 trained tasters, who conducted a sensory analysis using a descriptive test for cheeses based on the standard guidelines of hard and semihard cheeses (ISO standard 13299:2016). Panelists assessed odor and aroma attributes across 6 families: “lactic,” “vegetal,” “fruity-flowery,” “toasted,” “animal,” and “others.” Separately, they also scored the taste attributes of “sour,” “bitter,” “sweet,” “salty,” and “umami.” Additionally, they assigned scores for the quality of odor, aroma, taste, texture, and color. Data description analysis, spider charts, and Wilcoxon tests were used to compare milk type. Statistical significance was defined as $P < 0.05$ after correction for multiple comparisons. Cheese made with pasteurized milk tended to have higher mean values for the descriptors of “lactic” (2.75 ± 1.28 vs. 2.08 ± 0.98 , respectively) and “toasted” (1.76 ± 0.72 vs. 1.32 ± 0.49 , respectively) families, when compared with cheese made with raw milk ($P = 0.08$ and $P = 0.08$, respectively). There were no differences in salty and umami flavors, or in spicy and meat broth aromas and smells, characteristics typically associated with raw milk cheeses. In conclusion, no significant differences were observed between 60-d aged Manchego cheese made with raw or pasteurized milk using the descriptors collected in the standard guide. This work was supported by The Regulatory Council of Manchego Cheese, National Institutes of General Medical Sciences P20GM152304, Idaho Agricultural Experiment Station, and the University of Idaho P3R1 program.

Key Words: cheese, taste descriptor

2361 Controlled versus uncontrolled aging: Effects on the physicochemical, microbial, and sensory properties of Provolone dei Nebrodi PDO Cheese. G. A. Calandra Checco^{*1}, M. Caccamo², S. Ruta¹, C. Caggia^{1,2}, S. Petriglieri², G. Belvedere², G. Marino², and G. Licitra¹, ¹*University of Catania, Catania, Italy*, ²*CoRFiLaC, Ragusa, Italy*.

This study investigates the effects of collective aging under controlled conditions (CA) improves biochemical, microbiological, and sensory uniformity in Provolone dei Nebrodi PDO cheese while preventing early and late bloating defects, compared with uncontrolled aging (UCA) at the 6 selected dairies. A total of 288 cheeses (96 per production period) were aged for 240 d across 3 seasonal cycles: winter (W), spring (SP), and summer (S). Under UCA, temperature (T) ranged from 9.0 to 25.0°C , relative humidity (RH) from 46.0% to 65.0%, and airflow (AF) from 0.06 to 0.10 m/s, with seasonal trends that were higher in W and SP, lower in S, and inversely linked to production. These parameters were monitored using TTU 600 transducers, an ATMOS 22 anemometer, and a Grillo BEE system. In contrast, CA maintained stable conditions (T:

14 ± 1°C; RH: 80% ± 1%). Cheeses aged under CA exhibited higher fat content on DM (36.31% ± 1.14% to 56.34% ± 1.67%) compared with UCA (36.31% ± 1.14% to 54.16% ± 3.14%; $P < 0.05$) and higher protein content on DM (41.43% ± 1.84% to 55.65 ± 1.02% vs. 41.43% ± 1.84% to 52.32% ± 2.45%; $P < 0.0001$). pH stability was greater in CA (5.1 ± 0.1 to 5.5 ± 0.1) than in UCA (5.1 ± 0.2 to 5.9 ± 0.3; $P < 0.0001$). Proteolysis activity was higher in UCA, indicating increased protein degradation. Bloating defects occurred exclusively in UCA, with incidence rising from W (12.5% early, 14.6% ± 5.1% late) to S (29.2% ± 17.1% early, 31.3% ± 15.3% late), whereas no defects were observed in CA. Microbial dynamics varied significantly by season and dairy ($P <$

0.05), with lactic acid bacteria, yeasts, and molds initially higher in CA but surpassed by UCA after 60 d in W, 30 d in SP, and immediately in S. Sensory analysis ($P < 0.05$) revealed that UCA cheeses developed stronger aroma and flavor complexity but exhibited more textural defects, whereas CA ensured greater uniformity. These findings demonstrate that controlled aging mitigates seasonal variability, stabilizes biochemical and microbiological parameters, prevents fermentation-induced defects, and enhances sensory consistency in Provola dei Nebrodi PDO cheese.

Key Words: environmental fluctuation, pasta filata cheese, bloating defect

Dairy Foods: Chemistry—Posters

2362 Quantitative determination of A1, A2, and B β -casein in cow milk by capillary electrophoresis. G. Xian, J. Q. Wang, L. Meng, and N. Zheng*, *Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China.*

The increasing prominence of A2 milk (characterized by the absence of A1 β -CN) in the dairy industry stems from its purported health advantages, including enhanced digestibility and mitigation of gastrointestinal discomfort in susceptible individuals. However, comprehensive characterization of β -CNs compositional variability in commercial milk products remains limited, and robust analytical methodologies for precise quantification of these variants are critically underdeveloped. To authenticate and identify adulteration of A2 milk and its related products, this study extracted and purified β -CNs from milk and established a method for quantitative detection of B, A1, and A2 β -CN by capillary electrophoresis (CE). The optimal experimental conditions were finally determined through comparative tests of CE parameters: injection pressure of 0.5 psi, injection time of 10 s, and temperature of 38°C. The background electrolyte (BGE) comprised 50 mM disodium hydrogen phosphate, 140 mM citric acid, 4 M urea, and 0.2% HPMC (pH 2.9). The method validation showed that the baseline separation of β -CN was good, with excellent linearity ($R^2 > 0.9975$), recoveries (85.8%~101.2%) and intra-day and inter-day precision (RSD < 10%). Analysis of actual samples showed that commercially available A2 milks contained more than 90% of A2 β -CN and did not contain B β -CN, whereas the composition of β -CN in non-A2 milks varied considerably. Compared with other methods, this method is simple, rapid and achieves good baseline separation of B, A1, and A2 β -CN at the intact molecular level, which can meet the needs of quality testing of milk and milk products, and provides a technical means to identify the authenticity of milk.

Key Words: determination, β -casein, capillary electrophoresis

2363 Insight into differences in milk proteome from human and 8 dairy animal species for formula humanization. Z. Y. Ji^{1,2}, Y. Y. Yang², R. W. Han², J. Q. Wang¹, and N. Zheng*¹, ¹*Laboratory of Quality and Safety Risk Assessment for Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* ²*College of Food Science and Engineering, Shandong Technology Innovation Center of Special Food, Qingdao Agricultural University, Qingdao, Shandong, China.*

Human breastmilk fulfills the nutritional needs of infants and therefore is the best template for formula. Bovine milk has been extensively used as a substrate for infant formula, despite its distinctive nutritional composition compared with human milk. Interestingly, the importance and potential of low-yield non-bovine milk is a current issue, especially in infants who are allergic to bovine formulas. This study investigated the proteome differences in the whey, casein micelles, and milk fat globule membrane (MFGM) between human milk and the milk of 8 animal species (Holstein cow, buffalo, yak, camel, sheep, goat, horse, and donkey) using a proteomics approach based on liquid chromatography-high resolution mass spectrometry. Additionally, a laser diffraction particle size analysis was employed to compare the particle sizes of casein micelles and milk fat globules. The results showed that: (1) Multivariate statistical analysis revealed significant interspecies differences in 915, 780, and 1,170 proteins within whey, casein micelles, and MFGM, respectively. Compared with that from animal milks, human whey

exhibited significantly higher levels of digestive enzymes, including bile salt-stimulated lipase and β -galactosidase (fold change = 27.9–20,171.1, $P < 0.05$). Apolipoproteins (A, E, B) were more abundant in human milk casein micelles (fold change = 13.0–6,208.4, $P < 0.05$), whereas antioxidant enzymes (glutathione S-transferase, retinol dehydrogenase 11, catalase) were enriched in human MFGM (fold change = 3.2–137.2, $P < 0.05$). In addition, human milk contained almost no β -lactoglobulin in the whey and lower levels of α -casein (20.3%) in the casein micelles, which was significantly different from ruminant milk. (2) Characteristic high abundances of certain proteins were observed in specific animal milks. For example, whey acidic protein was identified only in camel milk whey. The abundance of lysozyme in casein micelles from donkey milk was significantly higher than that in ruminant milk and camel milk (Fold change = 13,307.9–228,209.6, $P < 0.05$). The abundance of Na⁺/glucose cotransporter in the MFGM of ruminant milk was significantly higher than that in human milk (fold change = 174.6–361.8, $P < 0.05$). (3) Human milk exhibited the smallest average particle size of casein micelles (124.87 ± 1.55 nm), followed by yak milk (149.70 ± 0.70 nm). Additionally, equine milk showed a greater quantity of smaller milk fat globules (0–5 μ m). Evidently, there are substantial differences in the proteome and physical characteristics between human milk and animal milk, which need to be taken into account in the improvement of infant formula.

Key Words: special milk, human milk, proteomics

2364 Analysis free and combined short-chain fatty acid profile in milk from Holstein cows, buffalos, yaks, goats, sheep, camels, human, donkeys and mares. S. C. Lu, Y. J. Qi, M. Q. Chen, N. Zheng, J. Q. Wang, and Y. D. Zhang*, *Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China.*

Short-chain fatty acids (SCFAs) were critical metabolites produced by the degradation and fermentation of dietary fiber, as an important component, not only play a significant role in the flavor, taste and nutritional value of dairy products, but also may have a profound impact on the metabolic health of the human body. SCFAs in dairy products are an important part of flavor in dairy products. Particularly, free short-chain fatty acids (FSCFAs) play multiply important roles in dairy products, which is not only the key component of dairy flavor, but also the core component to improve the nutritional value of dairy products and promote digestive health. In this study, we comparatively analyzed the composition of FSCFAs and SCFAs in 128 milk samples from Holstein cows, buffalos, yaks, goats, sheep, camels, humans, donkeys and mares by acidification injection and isopropyl esterification based on GC-MS, then, the content of the combined SCFAs (CSCFAs) is obtained by subtracting the content of the FSCFAs from the content of the SCFAs. The results revealed that the content of straight chain fatty acids in 9 animal milk was more abundant than that of branched-chain fatty acids. The SCFA profiles of Holstein cow, buffalo, yak, goat, and sheep milk were similar and contained more C4:0 and C6:0. The content of SCFAs was the highest in buffalo milk ($7,914.36 \pm 305.28$ μ g/mL), and yak milk second; sheep milk, goat milk, and Holstein cow milk were similar and decreased successively ($4,198.43 \pm 173.86$ ~ $5,514.77 \pm 224.29$ μ g/mL). In contrast, camel, human, donkey, and mare milk contained higher levels of C1:0, and the SCFAs of mare milk, human milk, camel milk, and donkey milk were lower ($1,957.71 \pm 121.14$ ~ $2,697.62 \pm 154.74$ μ g/mL). This study showed that the composition of SCFAs varies greatly between different animal milk. Although the

content of SCFAs in ruminant milk is higher than camel milk and monogastric animal milk, the proportion of FSCFAs of camel, human, donkey, and mare milk is significantly higher than Holstein cow, buffalo, yak, goat, and sheep milk. In which, donkey milk has the highest proportion of 59.41%, and the proportion of 7.99% is the lowest in sheep milk. Notably, camel milk was more similar to monogastric milk in the composition and content of SCFAs.

Key Words: free short-chain fatty acids, combined short-chain fatty acids, milk, GC-MS, species variation

2365 Development and validation of an LC-MS method for free monosaccharide analysis in milk from 8 species. Y. J. Qi, S. C. Lu, X. W. Zhao, M. Q. Chen, L. Song, J. Zhang, N. Zheng, J. Q. Wang, and Y. D. Zhang*, *Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China.*

Free monosaccharides are important carbohydrates in milk, providing both basic and bioactive nutritional benefits; however, the content and composition of free monosaccharides in milk from different species are still not well understood. The aim of this study was to develop a highly sensitive and accurate LC-MS method for the precise quantification of free monosaccharides in milk from 8 species, including human, cow, goat, sheep, yak, camel, mare, and donkey. The chromatographic conditions and mass spectrometry parameters were systematically optimized to ensure high resolution, minimal matrix effects, and low detection limits for all 8 target monosaccharides. The method was validated with excellent linearity ($R^2 > 0.996$), high recovery rates (94.18%–115.02%), and low coefficients of variation (<10%), demonstrating robustness across various concentrations. The compositional analysis revealed significant interspecies differences in monosaccharide profiles. Human milk was uniquely enriched in glucose ($4,262.98 \pm 246.49$ ng/mL) and fucose ($1,024.80 \pm 61.82$ ng/mL). In contrast, ruminant milk, such as cow and sheep, exhibited high levels of galactose ($1,803.56 \pm 94.63$ ng/mL and $1,230.31 \pm 52.33$ ng/mL, respectively) and mannose (375.24 ± 16.27 ng/mL and 55.81 ± 3.76 ng/mL, respectively). Principal component analysis (PCA) and a complementary stacked bar chart effectively visualized the clustering and relative distribution of monosaccharides among species, highlighting their metabolic and functional diversity. This study provides novel insights into the biological roles and evolutionary significance of milk monosaccharides. The developed LC-MS method offers a robust tool for advancing our understanding of milk composition and its implications for neonatal nutrition, dairy product innovation, and human health.

Key Words: free monosaccharide, milk composition, LC-MS

2366 Photoprotection of vitamin D₃ by a powdered form of lactoferrin with antioxidants from guava (*Psidium guajava* L.) leaf extract. A. Mora-Gutierrez*, M. T. Núñez de González, S. Woldesenbet, and R. Attaie, *Cooperative Agricultural Research Center, Prairie View A&M University, Prairie View, TX.*

Milk is commonly fortified with vitamin D₃ and is a rich source of riboflavin. Riboflavin is a photosensitizer for singlet oxygen formation. Such a photosensitized formation leads to vitamin D₃ degradation. Here, we studied the protective effect of a powdered form of lactoferrin with antioxidants from guava (*Psidium guajava* L.) leaf extract on the photosensitized degradation of vitamin D₃ in milk. The oxidation of 4,000 ppm vitamin D₃ in a model system of 12% water and 88% ethanol in the presence of 0 and 15 ppm riboflavin was determined by measuring the oxygen from the headspace with gas chromatography. A 15-mL

sample of this solution in a 20-mL serum bottle was air tightly sealed and stored in a 2,000 lx light box at 25°C. Adding the powdered form of lactoferrin with antioxidants from guava leaf extract (1.5 g/L) led to a significant 58% reduction ($P < 0.05$) in the photosensitized degradation of vitamin D₃ when compared with the control. The data from quenching and kinetic assays were evaluated by ANOVA and the means were separated using Tukey's test to determine significant differences. The singlet oxygen quenching rate by antioxidants from guava leaf extract is $24.31 \times 10^{10} M^{-1} \cdot s^{-1}$. The rate of singlet oxygen formation in a 20-mL serum bottle with 15 mL sample and 15 ppm riboflavin was $1.81 \mu M$ of oxygen/mL of headspace per hour. The powdered form of lactoferrin with antioxidants from guava leaf extract can be used to maintain nutritional quality of liquid milk products.

Key Words: vitamin D₃, lactoferrin, guava leaf extract

2367 Impact of laccase treatment on the functionality of milk protein concentrate and micellar casein concentrate. M. Akter* and P. Salunke, *South Dakota State University, Brookings, SD.*

Milk protein concentrate (MPC) and micellar casein concentrate (MCC) are crucial to increase the protein content and provide functionalities to the foods in which they are utilized. MPC and MCC are unable to provide critical functionality in certain products such as emulsions, yogurt, high heat stable products, and processed cheese products unless modified. Crosslinking enzymes such as laccase via oxidation and covalent bond formation have the potential to alter the physical and functional properties of MPC and MCC. The aim of this study was to determine the effect of laccase treatment of MPC and MCC retentates on their functionality. Three lots of MPC and MCC retentates were produced, and each replicate was divided into 3 portions. The MPC and MCC retentates were standardized to 12% proteins and treated with laccase enzyme at 3 levels: control (no laccase addition), low (2.5 units/g of protein), and high (5.0 units/g of protein). After enzyme addition, all the retentate treatments were incubated at 50°C for 15 min, and the enzyme was denatured using heat treatment at 80°C for 10 min and cooled to 4°C. Capillary gel electrophoresis (CGE) and particle size (PS) were done to determine the crosslinking. Various functional properties analyses were carried out, including heat stability (HS), foam properties, emulsion properties, and rheology. The CGE and PS analysis indicated crosslinking of proteins. The MPC samples had significantly ($P < 0.05$) higher PS and foam properties and significantly ($P < 0.05$) lower emulsion properties as compared with respective MCC treatments. The heat stability of MPC samples was significantly ($P < 0.05$) lower (633.5 s) compared with MCC samples (775.0 s). The enzyme treatment significantly ($P < 0.05$) affected HS and foam stability (FS). The HS and FS decreased significantly ($P < 0.05$) as the enzyme concentration increased, and the decrease in both HS and FS was greater in MPC samples. There was a significant ($P < 0.05$) increase in viscosity in MPC samples compared with MCC treatments. There was a decrease in viscosity in enzyme-treated samples compared with control samples. The study demonstrates that laccase treatment significantly affects and modifies the functionality of MPC and MCC.

Key Words: laccase, crosslinking enzyme, milk protein concentrate (MPC)

2368 Extraction of milk fat from ice cream waste using switchable solvents. J. Olatunde, N. Nkansah, K. Pizarro, and S. Martinez-Monteagudo*, *New Mexico State University, Las Cruces, NM.*

The industrial production of ice cream generates significant waste due to accidental spills, product changeovers, start-up operations, and equipment malfunctions. This waste contains food-grade fat that could potentially be recovered and reused in food and non-food applications. The aim of this work consists of extracting fat from ice cream waste using a tertiary amine (N,N-dimethylcyclohexylamine) as a switchable hydrophilicity solvent (SHS). For comparison, the fat was also extracted using the Soxhlet extraction. The ice cream waste was acquired from different industrial plants. Overall, the ice cream had a composition of $12.45\% \pm 1.21\%$ fat total, $4.12\% \pm 1.44\%$, and $42.78\% \pm 3.79\%$ total solid. The extraction time (3, 10, and 18 h), temperature (25, 40, and 60°C), and sample-to-solvent ratio (1/3, 1/10, and 1/18 vol/vol) were evaluated according to a factorial design. The extraction yield increased with the sample-to-solvent ratio and extraction time. The highest yield ($97.77\% \pm 2.01\%$) was obtained after 10 h of extraction at 60°C . On the other, the recovered fat from ice cream waste was significantly higher than the recovered fat obtained from Soxhlet extraction (14% and 12%, respectively). An incomplete conversion of CyNMe2 into bicarbonate salt appears to be responsible for overestimating the extraction values of total lipids. Overall, increasing the temperature resulted in higher values of extraction yield, regardless of the sample-to-solvent ratio. The outcomes of this work demonstrate the ability of CyNMe2 to extract fat from ice cream waste. However, fundamental extraction kinetics should be further investigated, and such knowledge can be used in process design and large-scale experiments.

Key Words: ice cream waste, extraction, milk fat

2369 Physicochemical properties of model sauces stabilized by heat-modified whey protein particles. C. Su*, M. Lin, and B. Vardhanabhuti, *Division of Food, Nutrition, and Exercise Sciences, University of Missouri, Columbia, MO.*

Global sauce markets have shown consistent growth over the past 5 years. Most sauces are emulsified foods containing 10%–60% fat at pH 3–5. The clean label trend presents an opportunity to replace traditional emulsifiers and stabilizers with dairy proteins. This study investigated the emulsification properties of heat-treated whey protein concentrate (WPC) particles in model sauces at pH 5, evaluating the effect of protein, oil, and salts concentrations. Whey protein particles were obtained by heating WPC (12% wt/wt protein, pH 5) at 85°C for 20 min, followed by dilution and gel disintegration through stirring and sonication. Emulsions were prepared at varying concentrations of protein (2%–5% wt/wt protein), oil (10–50% wt/wt), and salts (0 or 200 mM NaCl and 20 mM CaCl_2). Characterization of emulsions included the measurements of droplet sizes, zeta potential, rheological properties, and stability. Data were evaluated by ANOVA with means separation. Emulsions with 10%–30% oil and 2%–3.5% protein showed low viscosity and poor stability. At higher concentrations (40%–50% oil and 3.5%–4.2% protein), emulsions achieved homogeneity and stability, though 5% protein led to lump formation. Zeta potential values ranged from -20 to -24 mV without salt, decreasing significantly to -14 mV with salt addition ($P < 0.05$). Mean droplet sizes varied between 2.83 and 14.2 μm , with significant reductions observed upon increasing oil content and salt addition. All emulsions displayed shear thinning behavior. Although increases in oil (40 to 50%) or protein (3.5% to 4.2%) did not affect flow behavior index ($P > 0.05$), they significantly enhanced consistency coefficient ($P < 0.05$). Notably, emulsions containing 3.5% protein and 50% oil demonstrated viscosity comparable to commercial sauces at swallowing shear rates. In conclusion, this study highlights the potential applications of WPC particles in clean label sauces.

Key Words: whey protein concentrate, emulsion, sauce

2370 Quantification of lactobionate and lactose in calcium lactobionate solution by high-performance liquid chromatography. Y. Lin*, V. C. K. Yeung², and H. Zheng¹, ¹*North Carolina State University, Raleigh, NC,* ²*California Polytechnic State University, San Luis Obispo, CA.*

Previous research demonstrated that lactobionate (LBN), the salt form of lactobionic acid (LBA), may be manufactured from lactose in the cheese whey permeate (CWP) via enzymatic conversion. LBN is a value-added food ingredient, which may increase the net value of milk and whey permeates, however, the quantification method of LBN is scarce in the literature. This study aims to document an HPLC method for quantifying LBN content in the “product slurry” (PS), which was obtained from CWP solution after the enzymatic treatment. The objectives were (1) to develop standard curves for LBA anion and lactose; and (2) to validate the HPLC method regarding the quantification of LBN anion concentration in the PS. A Waters IC-Pak ion-exclusion column and 0.45 mM sulfuric acid were used as the stationary and mobile phases, respectively. A refractive index (RI) detector was used for detecting the eluted molecules. A model mixture solution was made by mixing lactose and LBA standards. The mixture solution was used for testing the method’s effectiveness regarding analyst separation. Each sample was prepared with no less than duplicates. The peaks assigned to lactose and LBA anion showed slight overlap on the chromatogram. Deconvolution of the overlapping peaks was conducted using Gaussian fitting. This method improved measurement performance by minimizing integration errors, reducing baseline noise effects, and improving the distinction between co-eluted peaks. The standard curves of both analytes with a concentration range of 0.5–10 mM fitted in linear regressions with acceptable coefficients of determination ($R^2 > 0.99$). To assess the method validity, a previously manufactured Ca-LBN slurry was spiked with a known amount of LBA standard; the obtained recovery rate of LBA was $89.33\% \pm 0.00\%$. This method may be adopted by the industry for quantifying LBN. Although the developed method is reproducible and suitable for LBN quantification, further optimization is recommended to enhance the separation of co-eluted peaks assigned to lactose and anion of LBN. For instance, alternative chromatography columns and different elution solvents and methods may be practiced in the future.

Key Words: ion-exclusion chromatography, calcium lactobionate quantification

2371 Predicting mozzarella cheese meltability with spectroscopy and machine learning. P. Freire*, K. Prayitno¹, D. Freire², and C. C. Licon³, ¹*Department of Food Science and Nutrition, California State University, Fresno, CA,* ²*Jordan Agricultural Research Center, California State University, Fresno, CA,* ³*Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo, CA.*

Cheese meltability is a critical functional property influencing the quality and applications of cheese in food products. Although current methods exist for assessing cheese meltability, they are time-consuming, and the lack of a standardized approach limits their consistency, comparability, and practical application. This study aims to demonstrate using visible and near-infrared spectroscopy coupled with machine learning (ML) to assess mozzarella cheese meltability. We developed a portable sensor-based device and accompanying software that used a multichannel spectral sensor (350–1,000 nm), a temperature sensor, and a computer unit. Three types of commercial mozzarella cheese were analyzed: fresh ($n = 6$), low-moisture whole milk ($n = 8$), and low-moisture part-skim ($n = 4$). The cheese’s meltability was assessed using a modified Schreiber test, where a cheese sample was shaped into cylindrical disks and placed in the center of a Petri dish with a Schreiber scale. The change in the diam-

eter of cheese before and after melting in a convection oven at 232.2°C for 5 min was measured. Then, the variation (final ratio subtracted by initial ratio) was calculated and normalized by the sample's weight. This procedure was repeated 3 times. In total, 54 mozzarella cheese pieces were analyzed, with 3 readings taken from the sensor device for each sample before melting. Meltability data were analyzed using ANOVA, a Tukey's test for means comparison, and supervised ML algorithms such as random forest (RF), extreme gradient boosting (XGBoost), and multivariate adaptive regression splines (MARS) to develop predictive models. Tukey's test showed 5 groups of meltability ranging from (0.18–0.31 mm·g⁻¹). All predicted models were cross-validated. The results demonstrated that meltability could be effectively predicted using machine learning models trained on light sensor data in the visible and near infrared range. The XGBoost algorithm yielded the strongest predictive performance with a coefficient of determination (R²) of 0.74. The RF model provided moderate predictive capability with an R² of 0.62, whereas the MARS algorithm showed limited effectiveness with an R² of 0.13. This work highlights the feasibility and potential of using sensor fusion combined with machine learning techniques for reliable and real-time meltability assessment suitable for on/in/at-line processes.

Key Words: meltability, cheese, machine learning

2372 Production of lactulose from whey permeate using magnesium oxide as catalysts. K. Pizarro, N. Nkansah, J. Olatunde, and S. Martinez-Monteaugado*, *New Mexico State University, Las Cruces, NM.*

Lactulose (LAU) is one of the most studied lactose-derived products due to its commercial relevance. Lactulose is an isomer of lactose, commercially produced from purified lactose through either a combination of soluble catalysts (e.g., NaOH and KOH) with complexing agents (e.g., borates) or enzymatic synthesis via glycosyltransferases and glycosidases. We proposed an alternative production for lactulose directly from lactose streams using MgO/SiO₂ as heterogeneous catalysis. The catalytic isomerization of lactose was performed at 110°C for 2 h over MgO/SiO₂ with different MgO loads (10–40 wt.%). The formation of lactulose was evaluated in a 5% lactose solution (LA), sweet whey permeate (SWP), and acid whey permeate (AWP). Values of lactose conversion were 25%–31%, 55%–86%, and 43%–58% for LAS, SWP, and AWP, respectively, regardless of the MgO load. The highest yields were achieved with SWP, reaching up to 49.38% ± 1.01%, followed by LAS (16.06 ± 1.51%) and AWP (5.26% ± 0.51%). The highest selectivity toward lactulose was obtained in SWP (75.25% ± 9.03%) 30%-MgO/SiO₂. The isomerization of lactose over MgO/SiO₂ showed great potential for the synthesis of lactulose from SWP directly without adjustment of the pH. Further optimization of the reaction kinetics is needed to maximize the yield and selectivity of lactulose at different reaction conditions.

Key Words: lactulose, isomerization, whey permeate

2373 Effect of cold maturation on the milk acidification kinetics. L. Rey-Cadilhac^{1,2}, C. Zerrouk¹, T. Morvant¹, S. Labrie¹, and J. Chamberland¹, ¹STELA Dairy Research Center, Institute of Nutrition and Functional Foods (INAF), Department of Food Science, Université Laval, Québec, Canada, ²PEGASE, INRAE, Institut Agro, Saint-Gilles, France.

For some protected designation of origin cheeses, such as Comté (France), milk is stored between 12 and 18°C for less than 24 h rather than at refrigeration temperature (≤4°C) before cheese making. This

process, called cold maturation, improves the cheese milk quality by limiting the solubilization of β-casein and colloidal calcium phosphate, reducing the growth of psychrotrophic bacteria while increasing the diversity of milk microbiota, and generating growth factors for cheese starters from casein hydrolysis. It is also hypothesized that cold maturation could standardize the acidification rate and reduce cheese variability. Still, cold maturation remains inconceivable at the industrial scale for time and volume considerations. Consequently, this study investigates the contribution of different dairy microorganisms, following cold maturation, on milk quality and fermentability before cheesemaking, with the long-term objective of reproducing the benefits of cold maturation with modern standardization strategies. Sterile 2%-fat cow milks were inoculated at the same concentration with different raw-milk-isolated strains (11 bacteria, 7 yeasts), and matured at 12°C for 16 h. These milks were then pasteurized and inoculated with a mesophilic culture starter. A control sample was used with the same sterile milk, unmatured, and acidified with the same starter. The acidification was monitored using a spectrofluorometric method, with 96-well microplates, measuring the pH every 5 min for 24 h at 32°C, in triplicate. To eliminate bias due to differences in the initial milk pH (pH₀), the pH was normalized by expressing it as a ratio relative to pH₀ (pH/pH₀). The results show that the maximum acidification rate and the time required to reach this rate are significantly affected by cold maturation with certain strains compared with the control (*P* < 0.05). Furthermore, the maximum rate is influenced by the strain used (*P* < 0.019). These findings highlight the influence of specific strains and initial cold maturation on acidification kinetics. The analyses are ongoing to evaluate the impact of cold maturation on proteolysis and free amino acids.

Key Words: cheesemaking, fermentability, raw milk microbiota

2374 Production of A2 milk in buffalo and Girolando cattle. E. R. Campanha¹, J. B. A. Asseituno¹, M. E. R. Fortini^{1,2}, R. S. Conrado^{1,2}, J. V. A. S. Mendonça¹, A. E. Rocha^{1,2}, and L. M. Fonseca^{1,2}, ¹School of Veterinary Medicine, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, ²Laboratory of Milk Quality Analysis, School of Veterinary Medicine, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

Beta-casein (β-CN) is a milk protein with 2 main variants: A1 and A2. Some people have gastrointestinal disorders due to milk containing β-CN A1, mainly because of the formation of a bioactive peptide called betacasomorphin-7 from the digestion of β-CN A1. The aim of this work was to investigate raw buffalo and Girolando milk for presence of A2 or A1 β-CN. From May to August, 2024, 32 refrigerated bulk tank milk samples were collected from different buffalo farms, and 16 individual milk samples were obtained from Girolando cows at an experimental farm. Girolando is an official Brazilian breed obtained from crossbreeding Gyr and Holstein cattle. All samples were tested using a kit (MilkTest A2; Scienco Biotech), and the Girolando cows were genetically tested for β-CN genetic variants A1 and A2, using tail hair (PCR and detection by capillary electrophoresis in a commercial laboratory). Buffalo milk was analyzed using CombiScope Fourier-transform infrared spectroscopy (FTIR) equipment, which is composed of a LactoScope FTIR for compositional analysis and a SomaScope for SCC (Delta/PerkinElmer). Freezing point was obtained by thermistor cryoscope method (Laktron, Brazil). All buffalo milk samples were A2A2, with the following results (mean ± SD) of composition: fat (5.97 ± 0.56 g/100 g), protein (4.07 ± 0.25 g/100 g), lactose (4.91 ± 0.10 g/100 g), total solids (15.84 ± 0.67 g/100 g), solids nonfat (9.87 ± 0.22 g/100g), casein (3.30 ± 0.21 g/100 g), milk urea nitrogen (18.76 ± 6.79 mg/dL). Average freezing point was -0.559 ± 0.015°C, and SCC was 228,000 ± 91,000 cells/

mL. Seven Girolando cows (43.75%) were genetically confirmed as A2A2 β -CN genotype, and were correctly identified using the Kit A2; One Girolando cow was genetically identified as A1A1 (6.25%) and 8 were identified as A1A2 (50%). Our results indicate that buffalo milk is A2 milk, because water buffalo only have A2A2 genotype for β -CN, as previously reported. Similarly, Zebu breeds of cattle, as for example Gyr, have A2A2 genotype for β -casein. Nevertheless, Girolando breed may have individuals containing A1 variant for β -CN because of the Holstein origin of the crossbreed. Girolando selection to obtain A2A2 animals must be based on genetic markers or milk testing, whereas milk products from buffalo milk are naturally A2. Commercial ELISA tests are an alternative for monitoring A2 milk authenticity.

Key Words: A2 milk, buffalo milk, Girolando milk

2375 Preparation of whey protein/mogroside nanoparticles and its digestion and sugar regulation properties. Q. Ban¹, Y. Meng¹, Y. Jiang¹, X. Wang¹, J. Cheng¹, and M. Guo^{*2}, ¹College of Food Science, Northeast Agricultural University, Harbin, China, ²Department of Nutrition and Food Sciences, College of Agriculture and Life Sciences, University of Vermont, Burlington, VT.

The development of functional nanoparticles for bioactive compound delivery has garnered significant attention in addressing metabolic disorders such as obesity and diabetes. Whey protein isolate (WPI) was used to prepare whey protein and mogroside (Mog) nanoparticles with core-shell structure by urea induction (20 mM). Mogroside may interact with whey protein (WP) molecules via hydrogen bonds and hydrophobic interactions, allowing it to be encapsulated within the hydrophobic region of the WPI nanoparticles. Hyaluronic acid (0.1 mg/mL) modified WP nanoparticles embedded Mog (H0.1-WU20-M) had a more compact and stable structure. Hyaluronic acid formed an interfacial film on the surface of WP nanoparticles through non-covalent forces (hydrogen bonds, hydrophobic interaction, and electrostatic interaction), demonstrating better anti-digestion characteristics than WP nanoparticles, effectively embedding Mog (embedding rate 90.84%), achieving sustained release delivery and improving its bioavailability. The regulatory effect of nanoparticles on high-fat diet-induced obesity was further explored using a mouse model. The mice (HW-Mog) treated with H0.1-WU20-M showed a significant reduction in body weight, which was 28.22% lower than that of the high-fat group. It also exhibited reduced fat distribution, with subcutaneous fat content approaching that of the control group. The complex nanoparticles improved leptin resistance, regulated GLP-1 secretion, inhibited blood glucose levels, and enhanced glucose tolerance in response to a high-fat diet. Histological analysis of the intestine and 16S rDNA sequencing of intestinal flora indicated that H0.1-WU20-M significantly reduced inflammatory damage to the liver, intestine, and

adipose tissue. The data indicated that feasibility of using embedded mogroside in the HA-modified nanoparticles for regulating glycolipid metabolism and improving intestinal flora of rats.

Key Words: whey protein isolate, nanoparticles, core-shell structure

2517 The effects of soy lecithin on foaming behavior and digestion properties of infant formula model system. S. Zhang¹, Y. Jiang¹, Q. Ban¹, J. Cheng¹, and M. Guo^{*2}, ¹College of Food Science, Northeast Agricultural University, Harbin, China, ²Department of Nutrition and Food Sciences, College of Agriculture and Life Sciences, University of Vermont, Burlington, VT.

Soybean lecithin (SL) is a polar lipid commonly added in infant formula products. Due to its low molecular weight and high surface activity, SL acts as an emulsifier together with protein to change the interfacial composition and structure of infant formula emulsions, thus affecting their stability, foaming and digestive behavior. In this study, different levels (0–6%, weight in oil) of SL were used as the emulsifier to combine with the infant formula protein model system (IFPMS) in production of infant formula emulsion. Multispectral techniques were used to study the interaction between SL and IFPMS. Then, the infant formula emulsion contained different SL (0–6%) were prepared, the stability properties, foaming behavior, and digestive performance were also evaluated. Multiple spectroscopic results verified that the IFPMS-SL complex was formed through hydrogen bonds and hydrophobicity bonds. The stability of emulsion showed a trend of an increase followed by a decrease, 5% SL resulted in emulsion with the smallest particle size (437.00 ± 25.63 nm) and highest absolute charge (-31.96 ± 0.47 mV). The foam volume of the emulsion in the control group remained 3.97 ± 0.33 mL at 60 min, however, higher levels of SL (>4%) dramatically decreased the foam volume of the emulsions and promoted the foam disappearance within 15 min. The higher interfacial pressure and lower dilatational modulus indicated that SL may replace the protein and change the composition at air-water interface. Furthermore, capillary electrophoresis photograph showed that the casein fraction in the foam phase was gradually decreased, reaching the lowest value of 5.93% at 5% SL, further revealing that casein was replaced by SL during foaming process. Soybean lecithin significantly improved the final free fatty acid release during in vitro digestion, and smaller particle size and higher net charge of digestive products can stabilize the emulsion and prevent flocculation. The results of this study may provide valuable information to formulate infant formula products for improving foaming properties.

Key Words: soy lecithin, infant formula protein model system, foaming behavior

Dairy Foods: Microbiology—Posters

2376 The effect of infant formula with milk fat globule membrane on *Bifidobacterium longum* ssp. *infantis* secretome and its subsequent effect on goblet cell barrier integrity. C. Miller* and R. Jimenez-Flores, *The Ohio State University, Columbus, OH.*

The milk fat globule membrane (MFGM) is a component of milk that is essential for infant gastrointestinal tract (GIT) and digestive system development. *Bifidobacterium longum* ssp. *infantis* is an important inhabitant of the infant microbiome and can effectively metabolize milk components to promote healthy GIT development. *Bifidobacterium infantis* can produce exopolysaccharides (EPS), which are used as protection through the harsh environments of the GIT and as fuel to promote cross-feeding networks in the microbiome. Interaction between the MFGM and *B. infantis* increase cell viability and function including promotion of bacterial adhesion to the epithelial layer for colonization. Separately, these 2 components have shown prevention of GIT permeability when challenged. However, the indirect effect of *B. infantis* metabolites from the use of MFGM as part of the fuel source on GIT permeability is not well established. Our hypothesis is that *B. infantis* will use digested MFGM to produce metabolites that further benefit the mucosal barrier of intestinal goblet cells. Infant formulas supplemented with MFGM underwent modified in vitro digestion. The digesta was then added to *B. infantis* growth media to measure growth patterns. The cell free supernatant (CFS) from all *B. infantis* growth conditions were collected at 4 time points to measure EPS production using phenol sulfuric acid total sugar assay and DNS reducing sugar assay. These CFS were used to treat HT29-MTX intestinal goblet-like cells on d 21 of confluency. The trans-epithelial electrical resistance was measured and protein extracted using RIPA lysis buffer for mucin and trefoil factor protein analysis with slot blot. Statistical ANOVA with post hoc analysis was performed in R. The results from this project show significant increase in EPS production with the greatest MFGM addition at growth phase ($P < 0.05$). Trans-epithelial electrical resistance and protein production were greater than the negative control. These findings suggest *B. infantis* may utilize infant digesta with MFGM in the GIT to aid barrier integrity.

Key Words: digestion, infant microbiome, intestinal permeability

2377 The main *Salmonella* serotypes identified in raw milk produced in the Brazilian Amazon region. J. C. Ribeiro-Júnior^{1,2}, F. L. Nunes^{2,3}, I. F. Alves³, M. A. C. Batista², J. P. C. da Silva², C. A. Nascimento², C. M. O. Lobo², A. A. Alfieri⁴, and L. B. S. B. da Costa¹, ¹The Ohio State University, Columbus, OH, ²Universidade Federal do Norte do Tocantins, Araguaína, Tocantins, Brazil, ³Agência de Defesa Agropecuária do Tocantins, Palmas, Tocantins, Brazil, ⁴Universidade Estadual de Londrina, Londrina, Paraná, Brazil.

Salmonella spp. is an enteropathogen frequently isolated in Brazilian raw milk. Research on these microorganisms is often limited to the genus, which does not allow epidemiological investigation of serotypes of special importance for public and animal health, such as *S. Enteritidis* and *S. Typhimurium*. This research aimed to verify the presence of *Salmonella* spp. in 80 raw milk samples produced in northern Brazil (Legal Amazon region), and to identify the main serotypes to support an epidemiological investigation. The samples were collected aseptically between August and October 2024 from 4 milk plants, with 20 samples taken from each plant. They were transported under refrigeration to the Food Microbiology Laboratory of the Federal University of Northern

Tocantins, where they were immediately processed. Qualitative analysis of *Salmonella* spp. was performed according to ISO 6579:2002/Amd 1:2007 and confirmed by PCR for the *invA* gene. From each milk plant, 2 isolates were randomly selected for typing by real-time PCR using the Check & Trace *Salmonella* 2.0 kit, each from a different milk sample. Of the 80 samples evaluated, 41 (51%) were positive for *Salmonella* spp. In total, 842 suggestive isolates were recovered, of which 165 (20%) were positive for the *invA* gene. In real-time PCR, it was possible to identify 7 different serotypes, with *S. Panama* present in 2 isolates from 2 different milk plants, and *Salmonella* Mbandaka, Bovismorbificans, Abaetetuba, Bredeney, Newport, and Infantis present in different samples. Given the wide variety of serotypes found in the samples, their known pathogenicity, and the potential for environmental or fecal contamination of milk, dairies must enhance the quality and safety of raw milk to mitigate risks in the dairy product chain. The study emphasizes the need for dairies to strengthen monitoring and control measures, such as pasteurization, to improve milk safety and protect public health.

Key Words: enteropathogen, *Salmonella* Panama, *Salmonella enterica*

2378 High occurrence of *Staphylococcus aureus* with potential for toxic shock syndrome toxin-1 synthesis and methicillin resistance in Brazilian raw fluid milk. J. C. Ribeiro-Júnior^{1,2}, F. L. Nunes^{2,3}, I. F. Alves³, A. L. Nascimento², J. F. Oliveira², C. F. S. Pereira², C. A. Nascimento², C. M. O. Lobo², A. A. Alfieri⁴, and L. B. S. B. da Costa¹, ¹The Ohio State University, Columbus, OH, ²Universidade Federal do Norte do Tocantins, Araguaína, Tocantins, Brazil, ³Agência de Defesa Agropecuária do Tocantins, Palmas, Tocantins, Brazil, ⁴Universidade Estadual de Londrina, Londrina, Paraná, Brazil.

Staphylococcus aureus is one of the main organisms causing mastitis in dairy herds. Depending on its pathogenic potential and antimicrobial resistance, the infection can persist in a chronic and recurrent form, leading to economic losses. Furthermore, quantifying this microorganism can indicate the hygienic quality of food handling and, when enterotoxigenic, it can compromise the safety of consumption throughout the entire production chain. This study aimed to quantify *S. aureus* in 80 samples of raw milk produced in the northern region of Brazil from August to October 2024. Serial decimal dilutions were performed and plated on Compact Dry XSA. The isolates were recovered and subjected to DNA extraction to investigate the presence of the *tst* gene, which encodes the synthesis of toxic shock syndrome toxin-1 (TSST-1), and the *mecA* gene, which can mediate methicillin resistance (MRSA). The colony count showed great variation in the 80 samples, ranging from 2 and 7.09 log cfu/mL, with an average of 6.04 (± 6.36) log cfu/mL. Of the total, 20 (25%) and 57 (71%) of the samples presented *S. aureus* counts higher than 6 and 5 log cfu/mL, respectively. A total of 1,017 isolates were recovered from all 80 milk samples. Of these, 597 (59%) were positive for the *tst* gene and 99 (10%) for the *mecA* gene. Of the milk samples, 81% (65/80) and 55% (44/80) had at least one *S. aureus* isolate positive for the *tst* and *mecA* genes, respectively. Among the MRSA, 72% (71/99) were also simultaneously positive for the *tst* gene. Therefore, high counts of *S. aureus* were detected, with a significant portion with the potential to synthesize TSST-1 and be characterized as MRSA. Under conditions for expression of these genes, these microorganisms can compromise the quality and safety of milk and dairy herds. These preliminary findings suggest the need for increased epidemiological surveillance, identifying critical points in the production chain, and

implementing measures to reduce its occurrence in mastitis and raw milk from dairy farms.

Key Words: methicillin-resistant *Staphylococcus aureus* (MRSA), toxic *Staphylococcus aureus*, toxic shock syndrome toxin-1 (TSST-1)

2379 Psychrotrophic proteolytic bacteria in the industrial slicing of mozzarella cheese. J. C. Ribeiro-Júnior^{1,2}, D. A. dos Santos², F. L. Nunes², M. A. C. Batista², K. T. F. da Silva², C. A. Nascimento², C. M. O. Lobo², A. A. Alfieri³, and L. B. S. B. da Costa¹, ¹The Ohio State University, Columbus, OH, ²Universidade Federal do Norte do Tocantins, Araguaína, Tocantins, Brazil, ³Universidade Estadual de Londrina, Londrina, Paraná, Brasil.

Microbiological contamination in industrial processes can lead to food spoilage, reduced shelf life, and various technological and sensory issues. This study aimed to assess the microbial diversity of proteolytic psychrotrophic (PP) bacteria before and after the mozzarella cheese slicing process. Ten mozzarella samples, produced and sliced by automated equipment at a milk plant in Brazil, were evaluated. Psychrotrophic counts were performed after serial decimal dilutions and inoculated on plates incubated for 10 d at 7°C. All recovered isolates were purified and subcultured on milk agar plates supplemented (1:9) with reconstituted (10%) skim milk powder and incubated at 7°C for 10 d for PP identification. The isolates showing proteolysis in the phenotypic assay underwent DNA extraction and internal transcribed spacer (ITS) amplification. These amplicons were subjected to restriction using the enzyme *Hha*I. The amplification profiles of the ITS region and their respective restrictions were used to build genetic similarity dendrograms. One representative of each cluster (60% similarity) was selected for identification by 16S rRNA partial sequencing. The mean psychrotrophic counts were 4.23 (±4.3) and 3.81 (±3.84) log cfu/mL for the samples before and after slicing, respectively, with no significant difference ($P > 0.05$). In total, 439 isolates were analyzed in the phenotypic assay: 228 before and 211 after slicing. A total of 47 (21%) isolates PP were identified, originating from 6 (60%) samples before the slicing; whereas 53 (25%) PP isolates were recovered from 8 (80%) sliced cheese samples. Before slicing, *Lactobacillus delbrueckii* (45/47) and *Bacillus* spp. (2/47) were the only PP species identified. Species such as *Staphylococcus* spp. (11/53), including *S. succinus* (4), *S. equorum* (2), and *S. hominis* (1), as well as gram-negatives like *Enterococcus faecalis* (1) and *Klebsiella pneumoniae* (1), were also identified. These findings suggest that even under automated and sanitary industrial conditions, PP may compromise the quality of the sliced cheese. Therefore, it is recommended to intensify procedures such as handler training and regular, and thorough cleaning of the automatic slicer.

Key Words: manipulation, cheese quality, *Staphylococcus* spp.

2380 Effect of a prebiotic galacto-oligosaccharides mixture on the growth of probiotics. P. Consolo*, S. Ruiz-Ramirez, and R. Jimenez-Flores, The Ohio State University, Columbus, OH.

Galacto-oligosaccharides (GOS) are prebiotics that positively affect the gastrointestinal microbiome, boosting growth of functional bacteria in the human gut. However, little is known of the quantitative effect of GOS on mixed bacteria growth. Our objective was to study whether a prebiotic mixture containing β -galactosidase derived from *Lactobacillus helveticus* OSU- PECh-4A had a prebiotic effect comparable to commercially used GOS products. We used 3 carbohydrate sources on modified de Man, Rogosa, and Sharpe (MRS) broth: lactose (LAC), a commercial GOS supplement (COM), and a GOS mixture produced using Beta-galactosidase isolated from *Lb. helveticus* OSU-PECh-4A (LHGOS), this last one produced in our laboratory. We evaluated the optical density (OD_{600nm}) and growth rate within the broths for *Limosilactobacillus reuteri*, *Lactobacillus rhamnosus*, *Lactococcus lactis*, *Bifidobacterium longum* ssp. *infantis* for 22 h at 37°C. The OD and maximum growth rate were higher for all strains in lactose as compared with oligosaccharides as the sole carbon source. *Limosilactobacillus reuteri* showed a preference toward COM having a difference in maximal growth rate $>0.1 \mu_{\max}$ h⁻¹ and OD ~2 with LHGOS and 3 for COM. The difference in maximum growth rate and OD of the different strains are shown in Table 1. *Lactobacillus rhamnosus*, *B. longum*, and *L. lactis* grew at a similar rate in the MRS broths with LHGOS and with COM. The LHGOS mixture showed a prebiotic effect comparable to the commercial product, with the advantage that being produced by a lactic acid bacteria, this enzyme may be of lower cost, presenting a significant contribution to the future of prebiotic products derived from probiotic-derived enzymes.

Key Words: prebiotic, optical density, *Lactobacillus helveticus*

2381 Biogenic amine degrading bacteria as a biotechnological tool for industry application. A. P. Rossetti, G. Perpetuini, A. Rapagnetta, O. F. Adesuyi, and R. Tofalo*, Department of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Teramo, Abruzzo, Italy.

Biogenic amines (BAs) are nitrogenous compounds derived by microbial decarboxylation of amino acids. They are generally accumulated in fermented foods such as dairy products. Their occurrence could be a food safety risk because they have vasoactive and psychoactive effects, making their reduction a priority in dairy processing. This study explores the ability of 16 *Lactocaseibacillus paracasei* strains, isolated from Parmigiano Reggiano cheese, to degrade BAs. Strains were tested for the presence of *hdc* and *tdc* genes, responsible for histamine and tyramine biosynthesis, respectively. The BAs degradation ability was assessed by HPLC in planktonic and biofilm-preadapted cells and the expression of the multicopper oxidase involved in BAs degradation was evaluated by qRT-PCR. Statistical significance was assessed using one-way ANOVA ($P < 0.05$), followed by Tukey's honestly significant difference post hoc test to determine significant pairwise differences between conditions. Three biological replicates and 3 technical replicates were performed to ensure data reliability. The *hdc* and *tdc* genes were absent in all strains. The BA degradation rate (%) was higher in biofilm-preadapted cells

Table 1 (Abstr. 2380). Difference in maximum growth rate and OD of the different strains

Bacteria	OD (maximum)				Maximum growth rate			
	LAC	LHGOS	COM	NO CARB	LAC	LHGOS	COM	NO CARB
<i>Lim. reuteri</i>	4.5	1.8	3.0	1.6	1.0	0.40	0.50	0.20
<i>Lcb. rhamnosus</i>	6.2	1.8	2.0	0.8	0.8	0.30	0.30	0.10
<i>B. longum</i>	6.3	1.9	2.3	1.0	1.2	0.25	0.25	0.20
<i>L. lactis</i>	2.4	1.8	2.0	1.9	0.4	0.25	0.30	0.25

than in the planktonic counterparts. In particular, *L. paracasei* LBH01 biofilm-preadapted cells achieved a cadaverine degradation rate of 50.2%, compared with 40.3% in planktonic cells. Similarly, histamine and tyramine degradation reached 33.2% and 36.2%, respectively, in biofilm-preadapted cells. The qRT-PCR analysis confirmed that the *mco* gene was more expressed in biofilm-preadapted cells than in planktonic ones. The highest overexpression was observed for LBH01 and LBP01 strains with a fold change of 11.9 and 9.4, respectively. These results suggested that the metabolic versatility of *L. paracasei* biofilm-preadapted cells could be implemented at the industrial level to enhance food safety, reduce regulatory concerns, and improve product quality.

Key Words: *Lacticaseibacillus paracasei*, biogenic amine, dairy product

2382 Enhancing protoplast formation of *Lactobacillus acidophilus*. R. C. Page¹ and K. J. Aryana^{*2}, ¹Louisiana State University, Baton Rouge, LA, ²Louisiana State University Agricultural Center, Baton Rouge, LA.

Lactobacillus acidophilus has been widely researched as a probiotic however, there is no adequate research on its protoplast formation. The aim was to optimize conditions for *L. acidophilus* protoplast formation. Freeze-dried *L. acidophilus* cells were suspended in 20 mM of HEPES buffer (pH 7) at different hyperosmotic conditions by adding sucrose (1 M, 1.5 M, and 2 M), to yield 10⁸ cells/mL. All *L. acidophilus* cells were treated with 125 µg/mL concentration of lysozyme and incubated at 37°C for 3 time points (30 min, 1 h and 2 h). All solutions before being combined (buffer, lysozyme, and cell suspension) were either set at 37°C by placing in a 37°C incubator for 10 min or (22°C) before the treatments mentioned above. Three fields were studied for each molarity, enzymatic/incubation time, and solution temperature combination by phase contrast microscopy. The independent variables included buffer molarity, lysozyme concentration (125 µg), exposure/incubation time, and solution temperature. The dependent variable was the survival rate of each strain following each treatment. Interactions among the factors were assessed using a 3-way ANOVA. Cell lysis and aggregation of *L. acidophilus* cells increased as incubation time increased. Cells suspended in 2 M HEPES buffer yielded a higher % of protoplast and reduced cell lysis when incubated for 2 h compared with the cells treated with 1 and 1.5 M. The *L. acidophilus* cell suspension, lysozyme, and buffer solutions adjusted to 37°C before incubation had a higher yield of protoplast after 2 h compared with solutions adjusted to room temperature (22°C). Buffer molarity and solution temperature both had significant effects on protoplast yield when incubated for 2 h. Protoplast formation decreased with prolonged exposure to lysozyme, indicating cell lysis. Buffer molarity played a significant role in this process, with 2.0 M buffer providing a protective effect, allowing for sustained protoplast formation even after prolonged exposure to lysozyme. This suggests that higher buffer molarity helps in maintaining cell integrity and minimizing lysis, thereby enhancing protoplast survival. These findings provide valuable insights for optimizing protoplast formation protocols, particularly in applications requiring prolonged enzymatic treatment.

Key Words: probiotic, acidophilus, protoplast

2383 Carbohydrate utilization patterns of heterofermentative lactic acid bacteria originating from raw milk. W. Y. Chueh*, C. O. Loomis, M. E. Henry, M. Wiedmann, and N. H. Martin, Cornell University, Ithaca, NY.

Among microbial contaminants in dairy products, heterofermentative lactic acid bacteria (Hetero-LAB) are particularly problematic and contribute to gas formation, off-flavors, and structural defects in cheese and other cultured dairy products. Their presence in raw milk and processing environments where they may contaminate dairy products after processing, highlights the need for effective detection methods to mitigate contamination risks, and ultimately reduce quality defects, economic losses, and consumer dissatisfaction. Their carbohydrate utilization contributes to gas formation, off-flavors, and texture defects in a wide range of product types, emphasizing the need to characterize their metabolic patterns and spoilage potential in dairy products. Therefore, the overall goals of this research were to develop a set of dairy-relevant Hetero-LAB and analyze their carbohydrate utilization patterns to determine the potential for dairy product spoilage. Based on our phylogenetic analysis, we hypothesize that closely related Hetero-LAB will exhibit similar carbohydrate fermentation patterns. To that end, we screened 102 raw milk samples for Hetero-LAB, resulting in 151 bacterial isolates that were characterized by sequencing the 16S rRNA gene for genus-level identification using SILVA ACT. Sequence types (STs) were determined using MEGA alignment and analysis in R. A randomly selected subset of isolates within each unique ST was further assessed for carbohydrate utilization using API 50 CHL. Overall, we identified 31 unique STs belonging to 9 distinct genera among the 151 isolates from raw milk, the most common of which were *Leuconostoc* (39.1%), *Weissella* (33.1%), and *Limosilactobacillus* (14.6%). The subset of 43 isolates selected for further characterization revealed distinct differences in carbohydrate utilization patterns among the isolates. Future work will combine carbohydrate utilization patterns and phylogenetic data to improve detection and tracking methods for Hetero-LAB in dairy environments.

Key Words: heterofermentative lactic acid bacteria, carbohydrate utilization

2384 Genomic diversity of prophages in *Pseudomonas fluorescens* species complex genomes. P. Vidigal^{1,2}, D. Cortessi¹, M. Alam¹, M. Lopez³, H. Hungaro⁴, and H. Mantovani^{*1}, ¹University of Wisconsin–Madison, Madison, WI, ²Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil, ³Universidad de Córdoba, Córdoba, Colombia, ⁴Universidade Federal de Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil.

Bacteria from the *Pseudomonas fluorescens* species complex are common contaminants in raw milk, contributing to spoilage and biofilm formation. Lytic bacteriophages are promising biocontrol agents to replace antibiotics in controlling bacterial biofilms. However, the limited availability of well-characterized phages infecting this complex constrains our understanding of their genomic diversity and applications. In contrast, genome mining enables the identification and characterization of prophages integrated within bacterial genomes, offering insights into phage-host interactions. This study analyzes prophage diversity and taxonomy across 531 genomes from 8 species of the *P. fluorescens* complex (*P. fluorescens*, *P. chlororaphis*, *P. corrugata*, *P. gessardii*, *P. jessenii*, *P. koreensis*, *P. mandelii*, and *P. protegens*). Prophages were identified using VIBRANT, VirSorter2, and PHASTEST, with CheckV assessing completeness. Prokka annotated prophage genomes, and taxonomic assignments were based on genomic similarity (VIRIDIC) and a proteomic tree (VipTree). We identified 1,692 prophage-like elements, many carrying host-associated genes that may influence bacterial fitness. Of these, 681 were classified as complete or high-quality and selected for genomic and taxonomic analysis. Comparative analysis with lytic *Pseudomonadales* phages revealed these prophages represent a previ-

ously unrecognized viral lineage, comprising 535 novel species and 422 novel genera, with substantial diversity even among prophages from the same bacterial species. The predicted prophage genomes encode 66,640 proteins, including 1,754 tail proteins, which could serve as molecular probes for detecting *P. fluorescens* contamination in dairy environments. Additionally, 11 tail proteins were identified as putative depolymerases with biofilm-disrupting potential, whereas 815 proteins were predicted as putative endolysins, offering a strategy for controlling *P. fluorescens* contamination in dairy products. These findings enhance our understanding of prophage diversity in the *P. fluorescens* complex and provide a foundation for biotechnological applications in food safety and biofilm control.

Key Words: prophage, biofilm, genome mining

2385 Validation of the limit of quantification for individual bacterial count (IBC) analysis by flow cytometry. R. S. Conrado^{1,2}, M. E. R. Fontini^{1,2}, E. H. P. Andrade¹, C. A. M. Penna¹, and L. M. Fonseca^{*1,2}, ¹*School of Veterinary Medicine, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil*, ²*Laboratory of Milk Quality Analysis; School of Veterinary Medicine, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil*.

The quality of milk, a highly nutritious food, can be significantly affected by microbial contamination, rendering microbial count a critical indicator of herd health and milk microbiological quality. During the last 2 decades, flow cytometry has emerged as a rapid and precise technique for analyzing individual bacteria count (IBC), but its validation is essential to ensure results reliability. This study aimed to validate the quantification limit for IBC according to the international standards (ISO 17025:2017 and ISO 16297:2020). The research involved evaluating key performance parameters, including precision (repeatability and reproducibility), accuracy (using reference materials and proficiency testing), and the limit of quantification (LQ). Milk samples with varying bacterial counts were analyzed using flow cytometry (BactoScan FC, Foss) and compared with the reference plate count method. Results of IBC were converted to cfu according to ISO 16297:2020. Linearity was established with a high coefficient of determination ($R^2 = 0.9901$), and precision was confirmed through repeatability (SD of $0.022 \log_{10}$) and reproducibility (SD of $0.02 \log_{10}$), both within acceptable standard limits. Method accuracy was validated using reference methods and control samples, showing strong correlation (0.92). The limit of quantification was determined to be 1,011 cfu/mL. This study confirms that flow cytometry is a reliable and accurate method for bacterial counting analysis, meeting regulatory requirements for milk quality assessment.

Key Words: individual bacteria count, milk quality, ISO 17025

2386 Development of a Monte Carlo simulation to quantify the influence of the bacterial community in bulk tank raw milk on the spoilage of HTST pasteurized milk. L. Kemmerling*, C. Qian, N. H. Martin, and M. Wiedmann, *Cornell University, Ithaca, NY*.

It is generally assumed that high temperature, short time (HTST) pasteurization eliminates vegetative bacterial cells found in raw milk, with the exception of bacterial spores and certain thermophilic organisms. However, because HTST pasteurization is designed to eliminate heat-resistant vegetative bacterial pathogens found in raw milk, not all food spoilage microorganisms are eliminated. Furthermore, it has been shown that even heat-sensitive spoilage microorganisms can survive HTST pasteurization with mathematically definable probabilities. Therefore, accurate quantification of surviving bacteria in raw milk helps assess

the value of high-quality raw milk and its impact on processed dairy product quality. This work modeled the die-off of the bacterial community found in raw milk during HTST pasteurization and subsequent growth under storage conditions at different supply chain stages utilizing a Monte Carlo simulation. A list of the 20 most common or relevant genera and their relative abundances in bulk tank raw milk (BTRM) was developed through a literature review of metagenomic studies looking at the bacterial community in BTRM. Alongside literature, expert elicitation (i.e., researchers with experience in dairy microbiology) was used to (1) curate the list of most relevant genera found in BTRM, and (1) estimate log reductions during HTST pasteurization for each relevant genus through 1-on-1 interviews. The experts were asked to assign each genus to 4 heat-resistance categories: (1) 0–1.5, (2) 2–4, (3) 4–6, and (4) 8–10 $\log_{10}$ cfu/mL reduction during HTST pasteurization, respectively. The category proportion estimates for each genus were averaged and used as the consensus for the model. The expected growth of the surviving cells during different supply chain stages was evaluated based on existing published growth data and modeled using a dynamic growth model. The model output, which was validated using shelf life data from dairy plants with low postpasteurization contamination, includes the proportion of simulated milk containers that reach $>6 \log_{10}$ cfu/mL throughout shelf life and the most abundant genera per container. This will provide insights into the genera found in BTRM that ultimately affect spoilage of HTST pasteurized milk.

Key Words: Monte Carlo simulation, bulk tank raw milk, spoilage

2387 Isolation, characterization and PCR detection of selected strains of lipolytic psychrotrophic bacteria from chilled raw milk. K. Yalaw^{*1,2}, X. Li¹, J. Lv¹, S. Zhang¹, N. Xie¹, Y. Wang¹, and X. Pang¹, ¹*Institution of Food Science and Technology, Beijing, China*, ²*Mekelle University, Mekelle, Tigray, Ethiopia*.

Dairy products are susceptible to contamination by microorganisms, such as psychrotrophic bacteria, which can degrade milk quality due to the production of heat-resistant enzymes. Detecting these bacteria early is crucial for preventing production losses in the dairy industry. This study aimed to isolate, characterize, and detect selected strains of psychrotrophic bacteria from raw milk, focusing on their psychrotrophic properties, lipase hydrolysis activity, optimal temperature, and PCR detection targeting the lipA gene that was responsible for fat and oils hydrolysis in milk and dairy products. The study employs culturing, extracellular hydrolysis test, design specific primer pairs, and molecular PCR amplification techniques for verification of 8 strains of the target gene isolates from the raw milk sample, which was among the data collection and analysis procedures employed in this study to prevent quality deterioration and production losses in the dairy industry. The findings in the research successfully indicate that the selected 8 strains isolated from stored raw milk are indeed psychrotrophic bacteria, with most exhibiting 6 strains lipase hydrolysis activity positive, 2 strains negative, and 1 negative control (NT) cannot express hydrolysis on the Malachite green agar plate, and the best optimal incubation temperature was 28°C. The designed primers show high specificity for only amplifying 6 lipase-positive, but cannot amplify for the 2 lipase-negative strains, and 1 NT, offering promise for early detection before milk processing. In conclusion, this study adds value by presenting a novel approach to detecting psychrotrophic bacteria in raw milk using PCR techniques. Future improvements may involve validating the methodology with a larger sample size and comparing the PCR method with advanced molecular techniques like qPCR and multiplex qPCR to assess its efficiency, specificity, and sensitivity.

Key Words: LipA, hydrolysis, psychrotrophic bacteria

2388 Effect of milk fat globule membrane and microbial community on serotonin production in a model system. A. Adams*, C. Miller, and R. Jimenez-Flores, *The Ohio State University, Columbus, OH*.

The intestinal microbiome is important for many body systems including the intestinal barrier. Probiotics, which are live microorganisms, can help balance gut microbiome dysbiosis and complications from an impaired barrier. Some probiotics, including lactic acid bacteria (LAB) commonly found in cultured dairy products, can produce serotonin from tryptophan and help regulate gut microbiota for those with certain gastrointestinal or mental health disorders. Additionally, milk fat globule membrane (MFGM) is a bioactive component in milk shown to greatly benefit the gut microbiome. It is hypothesized that the combination of probiotic LAB and addition of MFGM will enhance serotonin production by selected LAB. To understand this effect, 136 strains were screened for serotonin production and selected. From these, 4 cocultures (1-2, 1-3, 2-3, and 1-2-3) as consortia and 3 single strains of LAB (*Lactococcus lactis*, *Lactobacillus kefiranoferiens*, and *Lactobacillus helveticus*) were grown in milk permeate supplemented with 0.1% and 0.05% MFGM. The cultures were analyzed and compared with a control sample grown in milk permeate with no supplementation for serotonin production using spectrophotometry and Ehrlich's reagent. Based on the results, it is shown that the growth of these strains together with the supplementation of MFGM has a significant impact on the production of serotonin that appears to be synergistic. This research could present a method for improving mental and intestinal health.

Key Words: intestinal microbiome, serotonin, lactic acid bacteria

2389 Probiotic yogurt favorably shapes gut microbiota structure in obese mice. H. Shi^{*1}, T. Jetton², and J. Kraft^{1,2}, ¹*Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT*, ²*Department of Medicine, Division of Endocrinology, Metabolism and Diabetes, University of Vermont, Burlington, VT*.

Probiotic yogurt is reported to restore metabolic homeostasis via modulating gut microbiota, but its effect varies depending on the probiotic strain used. This study evaluated the impact of probiotic yogurt containing either *Lactobacillus plantarum* KABP-051 (LP), *Bifidobacterium* BB-12 (BB12), or both on gut microbiota in obese and lean mice. Five-week-old male and female wild-type (WT) mice (C57BL/6J, n = 30) and leptin-deficient obese (B6 Lep^{ob/ob}, ob/ob, n = 30) were both randomly assigned to 5 groups (n = 6/group): (1) control yogurt with starter cultures only (CON+), (2) LP yogurt, (3) BB12 yogurt, (4) combined LP+BB12 yogurt (COMB), and (5) no yogurt (CON-). Mice received 1 g/day of their respective yogurt (>10⁸ cfu/g). Body weight and feed intake were measured weekly to calculate the feed efficiency (g/kcal), and fecal samples were collected after 6 weeks for microbiota analysis. The ob/ob mice were 45% heavier than WT mice ($P < 0.0001$) and exhibited greater feed efficiency ($P = 0.0029$). In WT mice, LP and COMB yogurts increased α -diversity (observed richness, $P = 0.0004$; Shannon, $P = 0.0341$; Chao1, $P = 0.0002$) and β -diversity (Bray-Curtis, unweighted UniFrac, Jaccard, all $P = 0.001$). In ob/ob mice, yogurt treatments increased the β -diversity (all $P < 0.05$), regardless of the probiotic strain. Differentially abundant phyla in both WT and ob/ob mice included *Bacteroidetes* and *Actinobacteria*, whereas *Firmicutes* were enriched in WT mice only. BB12 and COMB yogurts increased the relative abundance of the *Actinobacteria* phylum and *Bifidobacterium* genus. The ob/ob mice had a lower *Firmicutes*/*Bacteroidetes* (F/B) ratio than WT mice (1.37 ± 0.33 vs. 1.56 ± 0.45 , $P = 0.04$). Compared with CON-, LP yogurt reduced the F/B ratio in WT mice (1.90 ± 0.62 vs. 1.05 ± 0.20 , $P = 0.0037$), whereas COMB yogurt further reduced the F/B ratio by 21% in ob/ob mice and 22% in WT mice. Collectively, probiotic yogurt containing both LP and BB12 was more effective than individual probiotic strains in differentially shaping gut microbiota structure in obese and lean mice, highlighting the potential utility of developing specific probiotic strain combinations to reduce metabolic disease risk.

Key Words: dairy, metabolic disease, gut microbial composition

Growth and Development: Posters

2390 Preliminary exploration of the effects of extended colostrum feeding on calf behavior, growth, and diarrhea. M. Tipton^{*1}, C. Velasquez¹, S. Bowman¹, J. Ahola¹, P. Carvalho¹, R. Moore-Foster², and C. Cramer¹, ¹*Department of Animal Sciences, Colorado State University, Fort Collins, CO*, ²*Department of Clinical Sciences, Colorado State University, Fort Collins, CO*.

Extended colostrum feeding (ECF) beyond 24 h of age can improve calf health and growth, yet the behavioral impacts are unknown. The primary objective of this pilot study was to determine if ECF from 0 to 14 d was associated with preweaning calf behavior. The secondary objective was to determine associations between ECF and average daily gain (ADG; kg/d) and diarrhea. Neonatal Holstein and Holstein-Limousin calves (n = 26) were enrolled after they received 4 L of maternal colostrum at birth and 4 L 8 h later, according to the dairy's existing protocol. Calves were transported 40 km to a university teaching and research facility at 1 d of age. Calves were randomly assigned to treatment (COL; fed milk replacer + 44 g Zinpro Premolac Boost colostrum replacer for the first 14 d, average Brix value = 14.5%) or control (CON; fed only milk replacer, average Brix value = 12.2%) upon arrival, blocked by breed type and sex (n = 15 females, n = 11 males). Electronic loggers were attached to each calf for 4 d to record lying time (min/d) and lying bouts (n/d) during wk 1, 2, 3, and 8 of life. Calves were weighed and ADG was calculated. Fecal scores were assigned to each calf using a 0–3 scale twice weekly; diarrhea was defined as a fecal score ≥ 2 . Lying behavior and diarrhea were analyzed using multivariable logistic regressions; ADG was analyzed using a multivariable linear regression. All models controlled for breed and sex and week was a repeated measure. Treatment was not associated with proportion of time spent lying (CON: 1.30 ± 0.04 ; COL: 1.31 ± 0.04 ; $P = 0.91$) or no. of daily lying bouts (CON: 3.17 ± 0.03 ; COL: 3.17 ± 0.03 ; $P = 0.99$). Treatment was not associated with ADG within the first 2 weeks of life (CON: 0.71 ± 0.06 ; COL: 0.76 ± 0.06 ; $P = 0.39$) or throughout the preweaning period (CON: 0.81 ± 0.06 ; COL: 0.81 ± 0.06 ; $P = 0.88$). 92% (12/13) of COL calves and 100% of CON calves had diarrhea at least once during the study period ($P = 0.18$). This pilot study did not find associations between ECF and lying behavior, ADG or diarrhea in preweaning dairy calves. However, this information can be used to refine sample sizes and measurements in future studies.

Key Words: ADG, health, lying behavior

2391 Effects of weaning age on the growth and metabolic status of dairy and dairy $\times$ beef crossbred calves. M. P. Pinkerton^{*1}, R. C. Neves¹, J. P. Boerman¹, H. W. Neave¹, E. M. Schafer¹, and J. A. Pempek², ¹*Purdue University, West Lafayette, IN*, ²*USDA-ARS Livestock Behavior Research Unit, West Lafayette, IN*.

Non-replacement dairy calves reared for beef may be weaned earlier than 8 wk of age due to cost, labor, and time. A growing proportion of this calf population is dairy $\times$ beef crossbreds, for which breed-specific requirements are undefined. The objective of this study was to determine the impact of weaning at 6 vs. 8 wk of age on the growth and metabolic health of dairy and dairy $\times$ beef crossbred calves. In 2 replicates, 32 bull calves (n = 16 Holstein [PB] and 16 Angus $\times$ Holstein crossbreds [CB]) were blocked by initial body weight (BW) and breed and randomized to be weaned at 42 (EW) or 56 d of age (CW). Calves were fed 2.8 L of milk replacer twice daily until d 35 (EW) or 49 (CW) when the morning feeding was removed for a 1-wk step-down until complete

weaning. All calves were individually housed with ad libitum access to a texturized calf starter. Starter intake was measured daily, and calves were weighed weekly; individual calf feed efficiency (gain:feed, FE) and average daily gain (ADG) were calculated. Blood was collected weekly from wk 3 to 8 and analyzed for whole blood glucose (GLU) as well as serum nonesterified fatty acid (NEFA) and insulin (INS) concentrations. Data were analyzed using PROC MIXED in SAS 9.4 with calf as the experimental unit; models included the fixed effects of treatment, breed, time, and their interactions, random effect of calf, and week as a repeated measure. Starter intake was higher ($P < 0.01$) in CW than EW calves during the week of step-down and complete weaning. However, from the week before step-down through weaning, FE was higher in EW (0.76 ± 0.96 kg/kg) compared with CW calves (0.62 ± 1.04 kg/kg; $P = 0.001$) and in CB (0.74 ± 1.04 kg/kg) compared with PB calves (0.63 ± 1.04 kg/kg; $P = 0.02$). There was evidence to suggest significant treatment $\times$ week interactions, whereby NEFA and INS were higher in CW than EW calves during wk 5 and 6, and GLU was higher in CW than EW calves during wk 5. No significant treatment or breed effects were apparent in overall BW or ADG around weaning. Although FE was higher in EW than CW calves, EW calves may have experienced increased metabolic stress at step-down and weaning.

Key Words: efficiency, crossbred calves, weaning

2392 Do milk replacer labels measure up? Characterizing management recommendations provided on milk replacer labels. M. M. Woodrum Setser^{*}, T. E. Da Silva, E. Allen, J. H. C. Costa, and K. C. Creutzinger, *University of Vermont, Burlington, VT*.

Milk replacers (MR) are frequently the primary nutrient source for young pre-weaned dairy calves. Labels on MR bags often provide other management recommendations beyond milk feeding strategies and volumes. Thus, the aim of this study was to characterize these recommendations on MR labels sold in Vermont. Labels were included from a convenience sample using an online search for the term 'calf milk replacer' and visiting livestock feed stores in the region. If a brand was identified in these searches, all calf MR sold by the same brand were also included. This search produced 52 calf MR from 12 brands. Feeding recommendations, including colostrum, calf starter, water, forage provision, and weaning recommendations were extracted from the labels. From this sample, 43% and 13% of products provided a colostrum volume (median [range]: 3.8 [2.8–3.8] L) and quality recommendation (IgG = 200 g/L), respectively. Suggested colostrum feeding time after birth was provided by 50.9% of products; of these, 81.4% provided specified a period (within 2 [1–6] h), 14.8% specified as soon as possible, and 3.7% stated it should be provided on d 1. Most (67.9%) products suggest when to introduce calf starter. Of these products, 77.8% provided a specific age (3 [2–4] d) and 19.4% suggest providing calf starter within the first week. Providing water to calves was suggested by 87.8% of products. Forage feeding recommendations were provided by 43.4% of products, where 95% of these state not to provide hay and the remainder state to provide it after 21 d of age. Only 37.7% of products provide feeding recommendations for cold weather, where 65% of these recommend consulting a nutritionist, and the remainder suggest feeding 50% more milk when cold ($<10^{\circ}\text{C}$). Lastly, 27% of products suggested a calf starter intake goal for weaning (1.4 [0.68–1.8] kg/d), and 20.8% suggested a weaning start age (48 [35–56] d). This study highlights the variety of topics and recommendations provided on MR labels. Further research

should evaluate the inconsistencies on MR labels and the impact on calf welfare and performance if managed based on these recommendations.

Key Words: preweaning, welfare, nutrition

2393 Impact of body weight at first parturition on production and reproductive performance. M. L. Simonds^{*1}, I. G. M. A. Santos¹, L. F. Brito¹, K. Kalbaugh², and J. P. Boerman¹, ¹Department of Animal Sciences, Purdue University, West Lafayette, IN, ²Agriculture Data Services, Purdue University, West Lafayette, IN.

The objective of this study was to further investigate the relationship between age at first calving (AFC) and body weight (BW) at calving, as well as how BW at calving affects milk production and reproductive success within first lactation. Data were collected from 3,333 first-lactation Holstein cows that calved between January 2021 and December 2022 from one commercial dairy farm in Indiana. 305 d milk production, BW at calving, AFC, sex of the calf, conception age in first lactation, pregnancy status by 200 DIM, and genomic predictions of breeding value for body size composite (BSC) were obtained from herd management software. The BW of cows after parturition were quartiled into groups: low (mean $\pm$ SD; 569 $\pm$ 25.6 kg), low-mid (612 $\pm$ 7.86 kg), mid-high (643 $\pm$ 10.2 kg), and high (707 $\pm$ 47.2 kg) for BW after parturition. Continuous variables were analyzed using a linear mixed model and binary variables (sex of calf, ketosis, mastitis, and metritis) were analyzed by logistic regression in SAS v9.4. The high BW group produced 635 kg more milk by 305 DIM than animals in the low BW group ($P < 0.05$). However, high BW group animals had the lowest predicted BSC of the 4 quartiles, the high BW group had a predicted BSC of -0.31 compared with the predicted BSC of the low BW group, which was -0.17 ($P < 0.05$). High BW group animals were 45 d older than low group animals at calving ($P < 0.05$). As BW at calving increased, the percent of female calves born decreased. Only 64.1% of calves born from animals in the high BW group were female compared with the low BW group where 74.4% of calves born were female ($P < 0.05$). The difference in AFC and proportion of female calves indicate that heifers that had higher BW at parturition required additional services to conceive and less sexed semen was used on this population. However, within first lactation there were no differences between the quartiles for DIM at conception (116.5 DIM; $P = 0.90$) or the proportion of animals not pregnant by 200 DIM (10.6%; $P = 0.35$), indicating that heifer conception success did not affect the cow's ability to conceive in the first lactation. Heifers that took longer to conceive had a greater BW at calving; however, conception within first lactation was not affected by BW at calving.

Key Words: primiparous, body weight, conception

2394 Assessment of the symbiotic bacterium *Clostridium butyricum* on the development of small intestine villi and the health of dairy calves. S. Vazquez-Flores¹, D. Galindo^{*2}, P. Flores-Martinez², F. Sanchez-Martinez², and S. S. Morales-Martinez³, ¹Tecnologico de Monterrey, Queretaro, Queretaro, Mexico, ²Huvepharma, Sofia, Bulgaria, ³GTH, Torreón, Coahuila, Mexico.

A comparative investigation of symbiotic bacterium *Clostridium butyricum* (Clb) on the development of intestine villi and health in dairy calves. The observational parameters were: frequency and duration of diarrhea, diseases, enteropathogen prevalence, feed intake, and growth. The experimental design included randomized allocation of treatments within blocks comprising 24 female and one male calf, with 6 repetitions. The control group (C) received no Clb, whereas the treatment group received 4 g of Clb in milk. Male calves were humanly

ethanized at 28 d old. The study spans 120 d and is ongoing. Calves were fed according to an age-specific regimen, at 13° Brix: 0–7 d, 2.5 L am/pm; 8–15 d, 4 L am/pm; 16–35 d, 6 L am/4 L pm; 36–46 d, 3 L am/2 L pm; and 47–57 d, 3 L am. Starter feed started at 150 g to 3 kg by d 57. Fixed effects were treatment, pathogen interaction, intestinal cells, and the random effect of calf within a treatment, all analyzed with inferential and nonparametric tests with JMP18.0.1 with a P -value of 0.05. Passive immunity, measured in Brix, differed between day-born and night-born calves, with consistently lower levels in the latter. Histopathological analysis revealed an increase in eosinophils for Clb and an increase in mitosis for C. Duodenal and ileal villi were longer for C, whereas jejunum villi were longer for Clb ($P = 0.08$, $P < 0.001$, $P < 0.04$, respectively). No significant differences in diarrhea rates were found, though *Cryptosporidium* prevalence was higher for Clb ($P = 0.04$). *Salmonella* spp. and *Rotavirus* infection rates were similar between groups, with lower occurrence of overall disease and fewer disease reports for Clb ($P = 0.04$). Starter intake was consistently and statistically higher for Clb from wk 1 to 8, except during wk 4 when calves were relocated from indoor individual cages to outdoor individual slots. Calves were weaned with an average starter intake of 2,464.3 $\pm$ 557.7 kg (Clb) and 2,232.8 $\pm$ 344.4 kg (C), with no significant differences in weight and height at 57 d. Microbiota analysis, pending for this abstract, is part of the study. The Clb demonstrated a lower overall disease rate, higher eosinophilic response, and fewer mitotic replications in the intestinal tissue, along with increased appetite throughout the study.

Key Words: symbiotic, *Clostridium butyricum*, dairy calves

2395 Performance and health of dairy calves fed organic vs. sulfate trace mineral forms in milk replacer during the pre- and weaning periods. E. Lopez-Bondarchuk^{*1}, J. V. R. Lovatti¹, T. E. da Silva¹, A. Keunen², A. J. Geiger³, and J. H. C. Costa¹, ¹University of Vermont, Burlington, VT, ²Mapleview Agri, Palmerston, ON, Canada, ³Zinpro Corporation, Eden Prairie, MN.

These randomized controlled trials assessed effects of trace mineral (Zn, Mn, Cu, Fe) form (organic vs. sulfate) on dairy calf performance and health during pre- (7L/d) and weaning (3.5L/d) periods. In experiment 1, 44 calves (25 bulls and 19 heifers) were enrolled at 0–3 d, began weaning at 63d, and were weaned at 70 d. Calves were randomly assigned to receive organic or sulfate trace mineral-formulated milk replacers, with all other components identical. In experiment 2, 127 bulls were enrolled at 0–3 d, began weaning at 42 d, and were weaned by 64d, receiving the same trace mineral treatments. Calf starter grain intake was recorded daily, and body weight (BW) weekly. In experiment 2, fecal scores were taken twice daily (a.m./p.m.) from 0 to 21 d (1 = solid to 4 = watery), using the higher score as the daily value. Diarrhea treatments were given from 0 to 21 d based on fecal scores, and respiratory treatments were administered at the onset of respiratory disease. Data were summarized weekly and analyzed using mixed models with repeated measures in R (v.4.4.2). Fecal scores were analyzed with cumulative link mixed models, and logistic regression assessed the probability of diarrhea and respiratory treatments. In experiment 1, no differences in BW were observed, but a sex-treatment interaction was observed for heifers (0 d; $P = 0.04$) and bulls (56 d; $P = 0.04$). Grain intake did not differ, but a sex-treatment interaction was observed ($P = 0.01$) for heifers (35 d; $P = 0.02$) and bulls (56 d; $P = 0.02$). No differences in ADG, but a sex-treatment interaction was observed ($P = 0.01$) for heifers (14 d, 35 d; $P = 0.04$). Feed efficiency (FE) did not differ. In experiment 2, no differences in BW, grain intake, ADG, or FE, but a FE-treatment-day interaction was observed (35 d; $P = 0.04$). Fecal scores and respiratory treatments did not differ, but diarrhea treatment probability differed ($P =$

0.01). Trace mineral form had minimal effects on calf performance and health; future work should investigate observed interactions.

Key Words: dairy calf, trace mineral, milk replacer

2396 Effects of fat content of high-protein milk replacer on plasma metabolite concentrations of dairy calves in summer and winter. R. Fukami^{*1}, N. Kobayashi², K. Murayama², M. Oba^{1,3}, and T. Sugino¹, ¹*Graduate School of Integrated Science for Life, Hiroshima University, Higashi-Hiroshima, Japan*, ²*Dairy Technology Research Institute, The National Federation of Dairy Co-operative Associations (ZEN-RAKU-REN), Nishi-shirakawa, Fukushima, Japan*, ³*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada*.

The objective of this study was to evaluate the effects of fat content of a high-protein milk replacer (MR) on plasma metabolite concentrations of Holstein calves in summer and winter. Fifty-four Holstein heifer calves born in summer ($n = 26$, 27°C) and winter ($n = 28$, 3.7°C) were fed one of the 2 MR (96% dry matter and 29% crude protein): (1) 21% fat and 4.7 Mcal/kg (LF; $n = 13$ for each season) or (2) 32% fat and 5.3 Mcal/kg (HF; $n = 14$ for each season), on a DM basis. The treatment MR were offered at 600 g/d (powder basis) from 7 to 13 d, 800 g/d from 14 to 20 d, 1,200 g/d from 21 to 41 d, 800 g/d from 42 to 48 d, and 600 g/d from 49 to 55 d. Calves were then weaned at 56 d of age. All the calves were fed a calf starter and chopped grass hay ad libitum. Blood samples were collected immediately before morning feeding. Maintenance energy requirement for thermal stress (ERT) was calculated as 2.01 kcal/kg EBW^{0.75} for each degree increase or decrease in ambient temperature outside the thermoneutral zone. Data were analyzed by PROC MIXED and correlation coefficients (r_s) were determined using Spearman's correlation method of JMP Pro 18. Only results for the peak milk feeding (21 to 41 d) are shown below. An interaction between treatment and season was observed for plasma alkaline phosphatase (ALP) concentration ($P = 0.04$); the LF calves had higher ALP concentration than the HF calves in winter but not in summer. Plasma glucose (GLU) concentration was lower in summer than winter ($P < 0.01$). The ERT for the HF calves had negative correlations with plasma concentrations of ALP ($P < 0.01$, $r_s = -0.68$), nonesterified fatty acid ($P = 0.03$, $r_s = -0.33$), and GLU ($P = 0.04$, $r_s = -0.32$) in summer. Plasma urea nitrogen concentration for the LF calves was higher than the HF calves ($P < 0.01$), and had a positive correlation with ERT in both summer ($P = 0.03$, $r_s = 0.37$) and winter ($P < 0.01$, $r_s = 0.66$). These results suggested that the combination of heat stress and high-fat MR may decrease lipid mobilization and gluconeogenesis and protein catabolism may be enhanced to generate energy for the LF calves when ERT increased.

Key Words: milk replacer, fat, metabolism

2397 Effect of birth season on Holstein calves' voluntary water intake. G. Antenucci, G. Visentin, Y. Vecchio, D. Cavallini, and A. Costa*, *Department of Veterinary Medical Sciences, Alma Mater Studiorum, University of Bologna, Bologna, Italy*.

Usually dairy calves do not have access to fresh water in the first d of life, even in summer when heat load is highly severe in certain challenging climatic areas. Although heat stress mitigation strategies, such as fans and sprinklers, are commonly present in the lactating cows' barn, in Italy newborn dairy calves are generally kept outdoor, and exposed to high temperature-humidity index (THI) in summer. In this study, we evaluated how the maximum THI (maxTHI) to which animals were exposed from 11 a.m. to 5 p.m. affected voluntary water intake in the first 21 d of

life (WI). We recorded daily WI in randomly selected female Holstein calves on an Italian commercial farm located in Po River Valley: 21 born in spring (mid-April to mid-June) and 22 in summer (late June to late August). Data were collected by the same operator and all calves were separated from the mother immediately after calving, provided with 6 L of colostrum in the first 6 h of life, kept in individual igloo pens, and fed twice a day with the same protocol and amount of milk replacer. In the 2 seasons average temperature between 11 am and 5 pm was 23.6 and 31.3°C, respectively, with maxTHI averaging 70.3 and 81.3. Regardless of season, by the first week of life, 20% of calves voluntarily drank some water. A one-way ANOVA was carried out with PROC GLM of SAS software v.9.4 for average daily WI and cumulative WI considering season as fixed effect. The effect was significant for both average (0.98 vs. 1.92 L, $P < 0.01$) and cumulative WI (16.45 vs. 26.77 L, $P < 0.01$). Although specific ethological data were not recorded, we systematically observed that buckets stimulated calves' curiosity and play behavior, e.g., blowing bubbles. Given that WI was non-negligible, even in spring, offering water to calves (an often overlooked practice) seems a low-cost, meaningful and positive measure for health and welfare, particularly in contexts with no early-life heat mitigation. Further research should be based on larger dataset, covering winter season and ethological aspects too. Investigating the effect of heat stress on number and length of daily bouts is advisable to calculate water requirement.

Key Words: water availability, heat stress, dairy cattle

2398 The impact of estimate conceptus weight on the nutrition and growth of heifers. N. Nishizawa¹, R. Harada^{*2}, T. Sugino³, and T. P. Tylutki⁴, ¹*Dairy Technology Research Institute, The National Federation of Dairy Co-operative Associations (ZEN-RAKU-REN), Nishi-shirakawa, Fukushima, Japan*, ²*The National Federation of Dairy Co-operative Associations (ZEN-RAKU-REN), Shibuya, Tokyo, Japan*, ³*Graduate School of Integrated Science for Life, Hiroshima University, Higashi-Hiroshima, Hiroshima, Japan*, ⁴*Agriculture Modeling and Training Systems (AMTS) LLC, Groton, NY*.

The objective of this study was to evaluate 3 different gravid uterine weight equations (CW) and how these impact target and predicted ADG. Equations were from (1) Fox et al. (1999; Fox99), (2) *Nutrient Requirements of Dairy Cattle*, 7th Edition (NRC, 2001) and (3) *Nutrient Requirements of Dairy Cattle*, 8th Edition (NASEM, 2021). Holstein heifers ($n = 62$), and their calves, from the ZEN-RAKU-REN research farm were weighed via scales at 149, 209, 237, and 265 d carried calf (DCC). Measured calf birth weight was used as an input in each CW equation. Table 1 illustrates the changes in body weight (BW) via scales and the differences in each estimate of CW from DCC 149 to the first calving day. The mature weight in ZEN-RAKU-REN research farm is 718 kg, the target first calving weight (82%–85% mature, conceptus free) is 589–610 kg. At DCC 265, the 3 equations relate to 85.9%, 84.9%, and 85.6% of maturity for Fox99, NRC2001, and NASEM2021, respectively. The heifers in ZEN-RAKU-REN research farms achieved 83%–84% of maturity at DCC 209, and it seems that the growth of conceptus free heifers does not increase after DCC 209. This illustrates target ADG must be reevaluated to ensure adequate heifer ADG. Given that retained energy, retained protein, and DMI are related to conceptus free BW, these differences also change formulation targets. Simulations performed with the dataset also suggest the heifer stops growing 4–8 weeks precalving. Thus, predicted CW plays a major role in evaluating and formulating bred heifer growth. Individual heifer growth data and sensitivity analysis of each equation will also be presented.

Key Words: heifer, growth, conceptus

Table 1 (Abstr. 2398). Body weight and predicted CW (kg) for DCC 149 to 279 for a 40-kg calf birth weight (average $\pm$ SD values in parentheses: average % mature)

Item	DCC				First calving day ¹	Mature ²
	149	209	237	265		
BW, ³ kg	568 $\pm$ 55.6	626 $\pm$ 52.3	639 $\pm$ 50.1	670 $\pm$ 52.4	606 $\pm$ 51.6 (84.4)	718 $\pm$ 76.0
Fox et al., 1999	10.9 (77.6)	25.7 (83.6)	37.6 (83.7)	53.7 (85.9)	62.9	—
NRC, 2001	0 (79.1)	27.4 (83.3)	44.0 (82.8)	60.4 (84.9)	68.0	—
NASEM, 2021	5.2 (78.4)	18.8 (84.5)	33.1 (84.4)	55.9 (85.6)	70.5	—

¹DCC: 278 $\pm$ 4.8 d.

²Fourth calving day: 62.9 $\pm$ 3.9 months of age.

³Scale weight: conceptus included (DCC 149–265), conceptus free (first calving day and mature).

2399 Effect of milk replacer and starter composition on intake, growth and intestinal permeability in Holstein-Angus crossbred calves. T. Chapelain¹, M. Kovacs¹, J. Martín-Tereso², D. J. Seymour^{1,2}, D. L. Renaud³, and M. A. Steele^{*1}, ¹Department of Animal Bioscience, University of Guelph, Guelph, ON, Canada, ²Trouw Nutrition R&D, Boxmeer, the Netherlands, ³Department of Population Medicine, University of Guelph, Guelph, ON, Canada.

The number of crossbred dairy calves has drastically increased over the past decade, but pre-feedlot nutritional recommendations remain scarce. This study investigated the interaction between pre- and postweaning diets with different energy substrates inclusion. Holstein-Angus male calves (n = 68) were assigned at 1 wk of age to 1 of 4 treatments in a 2 $\times$ 2 factorial arrangement: fat milk replacer (MR) + control starter (FC); fat MR + fat starter (FF); lactose MR + fat starter (LF); and lactose MR + control starter (LC). Fat and lactose MR were 29% and 18% fat, and 38% and 51% lactose (DM), respectively. Mixing rate was 150 and 135 g/L for fat and lactose MR, respectively, to balance energy and protein supply between treatments. Milk replacer was fed at 15% of arrival body weight (BW) until wk 3 and 18% thereafter until wk 6. Gradual weaning occurred from wk 6 to 10, with starter introduced at wk 5. Fat and control starter were 7% and 3% crude fat, and 36% and 37% starch (DM), respectively. Milk and starter intake were recorded daily, and BW was collected weekly. On wk 4 and 12 calves were orally dosed Cr-EDTA (0.1 mg/kg of BW) and blood was taken at 1, 2, 3, 4, 6, 8, 10 h post-dosing to assess intestinal permeability. Analysis of variance was conducted using treatment and calf nested in block as fixed and random effects, respectively. Milk replacer had no effect on BW or average daily gain (ADG), though calves fed fat MR had greater milk intake for the first 2 wks ($P = 0.01$). Calves fed the control starter had increased starter intake ($P = 0.02$), leading to higher ADG ($P = 0.04$) and a tendency for greater BW postweaning ($P = 0.06$). On wk 4, calves fed the fat MR had higher Cr blood concentration at 3, 4 and 6h ($P = 0.05$), but the area under the curve (AUC) was similar across treatments. On wk 12, calves fed the fat starter had increased AUC for Cr-EDTA compared with calves fed the control starter ($P = 0.01$). Although MR energy source had limited impact on performance, fat supplementation in starter reduced intake and postweaning growth, and increased Cr-EDTA recovery in blood.

Key Words: dairy beef, energy, intestinal permeability

2400 The effects of graded amounts of nicotinic acid on primiparous cow health, colostrum yield and quality of prepartum multiparous Jersey cows and growth in their subsequent calves. L. J. Valentine* and P. S. Erickson, University of New Hampshire, Durham, NH.

Nicotinic acid has been shown to increase the quality and quantity of colostrum, but a dosage has not been determined for Jersey cows. This study was conducted to measure the effects of nicotinic acid supplementation in a -40 DCAD diet on colostrum quality and quantity and the subsequent growth of calves. Thirty multiparous Jersey cows in a randomized complete block design were assigned to one of 3 treatments: 0 g/d, 8 g/d, or 16 g/d. Cows were blocked by calving date. Cows were moved to a pen fitted with Calan gates 28 d before the expected calving date. Cows were sampled weekly for urine, blood, and body weight (BW) was measured. At birth, calves were removed from their dams before suckling, weighed, and had blood sampled (for 0-h IgG) within 30 min of birth and were enrolled if their dams made ≥ 2 L colostrum. Twenty-seven calves were enrolled and remained on study until 6 wk of age with weekly body weight measurements. Calves were blocked by birth date. Data were analyzed using SAS with initial cow BW and parity as covariates. There was no difference in prepartum cow BW for 0, 8, and 16 g/d (542, 541, and 538 kg), respectively. Postpartum cow BW was not different for 0, 8, and 16 g/d (535, 536, and 533 kg), respectively. There was a quadratic effect for prepartum and postpartum ketone concentrations ($P < 0.04$), with the lowest value for the 8 g/d treatment (prepartum 0.54, 0.42, and 0.61; postpartum 0.52, 0.44, and 0.58 mmol/L, respectively). There were no differences in colostrum yield, colostrum Brix, initial and weekly BW, and skeletal measurements. There was a trend for a quadratic effect on calf blood ketone concentrations over the first 6 wk of life, with the lowest concentration for the 8 g/d treatment (0.18, 0.15, and 0.18 mmol/L, respectively). A correct dosage has not been determined.

Key Words: nicotinic acid, cow, colostrum, calf

2401 Do milk replacer labels measure up? Predicting calf growth based on milk replacer feeding recommendations. M. M. Woodrum Setser*, T. E. Da Silva, E. Allen, J. H. C. Costa, and K. C. Creutzinger, University of Vermont, Burlington, VT.

Nutrient availability is key to the health, performance, and welfare of dairy calves. Milk replacer (MR) is commonly used as the primary feed source for dairy calves, and most MR bag labels provide calf feeding recommendations. Thus, the objective of this study was to characterize MR feeding strategies on MR labels in Vermont and evaluate if they reach performance goals based on best practice recommendations. Labels were included from a convenience sample using an online search for the term “calf milk replacer” and visiting livestock feed stores in the region. If a product from a brand was sold in the region, the other products from the same brand were also included. This search produced 52 calf MR products from 12 brands. Label contents were extracted, including the nutrient composition, mixing instructions, and suggested

daily milk allowance. From this sample, 35 products from 11 brands provided sufficient information to estimate daily metabolizable energy (ME) from MR. The ME for each product was used to predict average daily gain (ADG) using a NASEM adapted model (CalfSim Software; go.uvm.edu/calfsim) for a standard calf born at 37 kg through 56 d of age, assuming thermoneutral conditions and free access to a standard calf starter (22% crude protein, pelleted). Predicted ADG by product was assessed on whether the calf would double birth weight by weaning (0.66 kg/d), a common growth goal in the dairy industry. Labels were sorted into 3 categories based on their suggested feeding rate from birth to weaning: (1) one rate (ONE; $n = 22$), (2) a low to high range of rates (RANGE; $n = 6$), and (3) rates based on calf age (PHASE; $n = 7$). Of the MR, none of ONE (mean $\pm$ SD: 0.38 ± 0.07 kg/d ADG) or RANGE (0.51 ± 0.07 kg/d ADG), and all of PHASE (0.86 ± 0.02 kg/d ADG) predicted an ADG at or above our goal. Although limited, these simulated growth results highlight the variation in recommended milk feeding rates for pre-weaned dairy calves across different products. Many MR suggest feeding levels that negatively affect calf welfare and performance, indicating misalignment between label instructions and scientific or industry standards.

Key Words: preweaning, welfare, nutrition

2402 The gastrointestinal microbial communities of weaned bull calves are negatively impacted by dam nutrient restriction during late gestation. M. K. Costello^{1,2}, J. C. McClure³, R. Amorin de Hegedus^{2,3}, E. G. Olson¹, E. A. Stoebe³, W. Li³, C. Thiago¹, L. L. Hernandez¹, E. A. French³, and S. C. Ricke¹, ¹University of Wisconsin-Madison, Madison, WI, ²Oakridge Institute for Science and Education, Oakridge, TN, ³USDA-ARS-DFRC, Madison, WI.

Nutrient restriction during late gestation was previously shown to negatively affect offspring growth and development. This study aimed to characterize the impacts of maternal nutrient restriction or overfeeding on the gastrointestinal tract (GIT) microbiome in weaned Holstein bull calves. Weaned bull calves born to nutrient restricted dams (LE calves, $n = 11$) and high-energy fed dams (HE calves, $n = 5$) were euthanized between 67 and 73 d of age. Calves were fed texturized calf starter ad libitum and were weaned at 48 d of age. Digesta samples and pH measurements were collected from 7 GIT locations: rumen, abomasum, duodenum, jejunum, ileum, cecum, and large intestines. We extracted DNA from samples using 2 different methods. The DNA extracted with DNeasy was used for amplicon sequencing of the V4 region on the 16S rRNA gene, run on the Illumina MiSeq platform. The DNA was extracted with a precipitation method to preserve fragment length for long-read metagenomic sequencing on an Oxford Nanopore Technology P2 solo. Short-read sequencing data were trimmed and taxonomically assigned in QIIME2-2023.9 and subsequently uploaded into R Studio for α and β diversity, core microbiome, ANOVA, ADONIS, and ANCOM analyses. Metagenomic data were taxonomically and functionally classified with Kaiju and EggNOG-mapper, respectively. The HE calves possessed more diverse large intestinal communities, whereas the LE calves had more diverse abomasal communities ($P < 0.05$). In the rumen digesta, HE had higher abundances of *Methanobrevibacter*, and *Eubacterium coprostanoligenes*, and *Monoglobus* were enriched in the cecum and large intestines digesta ($P < 0.01$). LE calves had higher abundances of *Bifidobacterium*, a starch-utilizer, in the rumen, abomasum, and cecum. More antibiotic-resistant genes were identified in the rumen contents of LE calves ($P < 0.05$), whereas more genes involved in fatty acid biosynthesis were identified in the rumen digesta of HE calves ($P < 0.05$). Our results indicate that maternal nutrient restriction negatively affects the GIT microbiome and microbial functional capacity of recently weaned

Holstein bull calves by promoting less efficient starch utilization in the foregut and reducing diversity in the hindgut.

Key Words: microbiome, gastrointestinal tract, calf

2403 Identification of early mammary ultrasound predictors for first-lactation performance. A. L. Vang^{*}, Z. Marohn, W. F. Frizarini, G. J. M. Rosa, J. R. R. Dorea, and L. L. Hernandez, *University of Wisconsin-Madison, Madison, WI.*

Ultrasound (US) imaging is widely used in large animals, but its application for predicting milk production via mammary gland assessment remains limited. This study aimed to identify early indicators of high milk production in dairy calves using US imaging. Holstein heifer pairs, matched by birthweight, were fed low (LM, 1.89 L/d, 22% CP, 15% fat, $n = 48$) or high (HM, 3.78 L/d, 27% CP, 20% fat, $n = 48$) milk replacer for 7 wk. Mammary parenchyma (PAR) and fat pad (FP) echogenicity and area were quantified from US images using QuPath software. Linear mixed models analyzed PAR mean echogenicity, PAR area, FP mean echogenicity, and FP area, including fixed effects of diet $\times$ wk (0, 1, 3, 5, 7, 9, 11), birth month, starter intake, body weight, and first lactation production (LP, HP), with calf as a random effect. The HM calves had increased body weight from wk 3 to 11 ($P < 0.050$) and larger PAR area at 11 wk ($P < 0.001$). Mean PAR echogenicity peaked at 5 wk and declined thereafter ($P < 0.001$). Mean FP echogenicity increased from 5 to 7 wk ($P < 0.001$), and FP area increased from wk 3 to 11 ($P < 0.001$), particularly in HM calves from wk 9 to 11 ($P < 0.050$). A linear model analyzed first-lactation energy-corrected milk (ECM) with diet, calving weight, average daily gain, starter intake, and 11-wk body weight, and PAR and FP areas. The ECM tended to be positively associated with PAR area ($P = 0.058$) and negatively with FP area ($P = 0.081$). A significant diet $\times$ production interaction for FP area ($P = 0.012$) and PAR mean echogenicity ($P = 0.003$) at 5 wk was observed. The LM/LP calves had increased FP area ($P = 0.038$) and greater PAR mean echogenicity ($P = 0.014$) than LM/HP. At 11 wk, a diet $\times$ production interaction ($P = 0.045$) and effect of production ($P = 0.048$) was observed for FP area, with HP calves having a reduced FP area. Mean FP echogenicity tended to be elevated in LP calves ($P = 0.075$). These findings suggest early mammary development, rather than weight or diet alone, may serve as a predictor of future milk production. The LP calves had greater PAR and FP echogenicity and larger FP area at 11 wk, warranting further investigation into molecular and histological mechanisms linking early mammary development to milk yield.

Key Words: ultrasound, mammary gland, development

2404 Extracting individual factors that influence starter intake and growth in dairy calves during pre- and postweaning. T. E. Da Silva^{*1}, J. V. Lovatti¹, D. L. Renaud², A. J. Keunen³, and J. H. C. Costa¹, ¹University of Vermont, Burlington, VT, ²University of Guelph, Guelph, ON, Canada, ³Mapleview Agri, Palmerston, ON, Canada.

Starter intake and growth in dairy calves are influenced by intrinsic animal factors, nutrition, management, and health status. This study aimed to disentangle and describe individual animal factors associated with starter intake and body weight in dairy calves during pre- and postweaning periods using a comprehensive database of 480 Holstein male calves. The database was derived from 4 experiments involving 12 nutritional intervention treatments. Variables evaluated for their potential impact on calf starter intake and body weight included (fixed effects): days on trial (from 1 to 84), initial body weight (47.9 ± 3.87 kg), serum total protein at arrival (5.6 ± 0.63 g/dL), calf source (4 sources),

metabolizable energy intake from milk replacer (ME; 3 ± 1.9 Mcal/day), and occurrences of diarrhea and respiratory disease. A generalized linear mixed model with a Tweedie distribution was used, incorporating random effects for study, treatment nested within study, animal nested within treatment and study, and calf source. The effect of days on trial was tested in linear, quadratic, cubic, and quartic polynomials (i.e., random regression model). A significance level of 0.05 was used to retain fixed effects in the model. For calf starter intake, days on trial, initial body weight, ME intake from milk replacer, diarrhea, and respiratory disease bouts were retained in the model ($P < 0.01$; RMSE = 0.24 kg; $R^2 = 0.84$). Similarly, for body weight, days on trial, initial body weight, ME intake from milk replacer, diarrhea, and respiratory disease bouts ($P < 0.05$; RMSE = 2.92 kg; $R^2 = 0.93$). Initial body weight and ME intake from milk replacer were positively associated with both starter intake and body weight. In contrast, diarrhea and respiratory disease occurrences were negatively associated with starter intake and body weight. These findings highlight the importance of nutritional management and health status in optimizing early life growth and starter intake in dairy calves.

Key Words: disease bouts, modeling, nutrition

2405 Diarrhea and fever prediction in Holstein calves using machine learning. J. Diavão^{*1}, A. S. Silva¹, S. M. Mendes², A. V. das Graças³, A. R. S. Loures⁴, S. G. Coelho², and M. M. Campos¹, ¹*Empresa Brasileira de Pesquisa Agropecuária - Embrapa Gado de Leite, Juiz de Fora, Minas Gerais, Brazil*, ²*Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil*, ³*Universidade Federal de Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil*, ⁴*Centro Universitário Presidente Antônio Carlos, Juiz de Fora, Minas Gerais, Brazil*.

The objective of this study was to evaluate the effectiveness of a machine learning algorithm in predicting diarrhea and fever in pre-weaned dairy calves, considering a set of rumination and activity data. The database was composed of variables obtained from 29 calves housed in individual pens, from birth to 60 d of age. One day after birth, each calf received an ear tag (EAR TAGS; Sensehub Dairy, Merck & Co. Inc., Rahway, NJ) and rumination and activity data were measured daily. Calves were considered sick by visual estimation when they presented diarrhea score ≥ 2 and/or fever $\geq 39.5^\circ\text{C}$ (using a portable digital clinical thermometer; G-TECH TH1027 Accumed, Joytech Healthcare, Zhejiang, China) with reading for 1 min in the rectum. Logistic regression was used to train the model to classify the calves, and the occurrence of disease was identified binomially (0 = healthy; 1 = sick) for data analysis. For data partitioning and analysis, 70% were used to train the model and 30% were used to test the model. The model evaluation metrics were accuracy, F1-score and recall. The model used considered fever or diarrhea as the dependent variable and rumination and activity data as predictor and independent variables. The data were analyzed using the RStudio statistical package. Both variables (rumination and daily activity) showed a significant effect ($P < 0.01$ and $P < 0.001$, respectively) on diarrhea and fever prediction. The model accuracy was 0.65, and 0.5672 to 0.7379 of confidence interval (95%), recall of 0.83 indicating that the model has a good ability to identify positive cases (sick calves) and F1-score of 0.69, indicating a good balance between precision and recall, and the test power was 70% ($P = 0.05$) Rumination and daily activity have been shown to be able to predict the occurrence of sickness calves. This suggests that changes in these behaviors may be indicators of health problems. The results of this study may help in making more efficient decisions in the health management of dairy calves, contributing to the improvement of animal welfare and farm productivity. In addition, to

improve the accuracy of the model, it is suggested that more repetitions need to be incorporated into the database to build a more robust model.

Key Words: animal health, early warning system, logistic regression

2406 Exploring how diarrhea, respiratory disease, and other variables impact feed efficiency in dairy calves. M. J. Vandewiel^{*1}, K. Broadfoot¹, A. Keunen², M. A. Steele³, and D. L. Renaud¹, ¹*Department of Population Medicine, University of Guelph, Guelph, ON, Canada*, ²*Mapleview Agri, Palmerston, ON, Canada*, ³*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada*.

The objective of this cohort study was to assess factors associated with feed efficiency in dairy calves, with a focus on diarrhea and respiratory disease. This study was conducted at a commercial calf rearing facility in Southwestern Ontario, Canada, and included 726 Holstein and 40 crossbred dairy calves, aged 3 to 10 d upon arrival, and housed for 84 d. Calves were weighed at arrival and weekly thereafter. Calves were fed milk replacer twice daily, receiving up to 520 g of powder mixed in 4 L per feeding, with a 2-wk step-down weaning period starting on d 49. After each milk feeding, refusals were weighed, and total milk intake was calculated. For solid feed, new feed was weighed at each feeding and refusals were weighed weekly. Fecal consistency scoring was performed twice daily for the first 28 d, and respiratory scoring was conducted for 84 d using the UC Davis respiratory scoring chart. A mixed linear regression model, controlling for arrival group as a random effect, was created to evaluate the association of feed efficiency with other measured factors. The mean ($\pm$ SD) feed efficiency from arrival until the end of the study period was 6.82 ± 0.88 kg of feed per kilogram of BW gain. The final model identified an interaction between diarrhea and respiratory disease that was associated with feed efficiency. Specifically, calves with 1 to 3.5 d with diarrhea and ≥ 2 d with respiratory disease required an additional 0.37 kg of feed per kg of BW gain ($P = 0.02$; 95% CI: 0.07–0.23) compared with those without diarrhea with ≥ 2 d of respiratory disease. Similarly, those with ≥ 4 d with diarrhea and ≥ 2 d with respiratory disease took an additional 0.29 kg of feed per kg of BW gain ($P = 0.05$; 95% CI: 0.01–0.58). Additionally, calves weighing > 50.7 kg upon arrival required an additional 0.15 kg of feed per kilogram of BW gain ($P = 0.05$; 95% CI: 0.001–0.298) compared with calves weighing < 45.3 kg. Holstein calves also required an extra 0.30 kg of feed per kilogram of BW gain ($P = 0.02$, 95% CI: 0.03–0.30) than dairy beef calves. This study provides the first step toward understanding feed efficiency in dairy calves, and how common risk factors can impact this metric.

Key Words: feed efficiency, dairy calf, diarrhea and respiratory disease

2407 Effect of probiotic fed in milk and starter grain during the pre-wean period on gut development, diarrhea, and gut microbiome in pre-weaned Holstein $\times$ Angus calves. M. J. Brasil^{*1}, J. L. Cardin¹, D. B. Vagnoni², K. E. Vagnoni², M. Macias-Rioseco³, S. Kim⁴, J. S. Van Kessel⁴, B. J. Haley⁴, and H. A. Rossow¹, ¹*Veterinary Medicine Teaching and Research Center, University of California, Davis, Tulare, CA*, ²*California Polytechnic State University, San Luis Obispo, CA*, ³*California Animal Health & Food Safety Laboratory, Tulare, CA*, ⁴*United States Department of Agriculture, Agricultural Research Service, Environmental Microbial & Food Safety Laboratory, Beltsville, MD*.

Administering probiotics can improve gut development and delay the onset of diarrhea in pre-weaned calves. This study evaluates the effectiveness of feeding a *Lactobacillus acidophilus* and *Lactobacillus casei*

probiotic supplement (PS; 1×10^9 cfu) in milk and starter grain once per day on blood β -hydroxybutyrate (BHB) and glucose (GL) concentrations, time to first diarrhea event, and gut microbiome metagenomic analysis. On a commercial calf ranch in California, pre-weaned Holstein $\times$ Angus cross calves age <48 h with serum total proteins ≥ 5.2 g/dL were randomly enrolled into 2 treatment groups: (1) control (CON; $n = 149$), and (2) probiotic (PRO; $n = 150$). Fifty mg/calf per day of PS were added to individual milk bottles in the a.m. feeding, and 50 mg/calf per day were top-dressed in starter grain until weaning at 60 d of age. Calves were housed individually in wooden slatted hutches. Starter grain was provided beginning at 14 d of age, and 2 L of milk replacer was fed twice a day. Blood samples were collected from approximately 50 calves per treatment at 7, 21, 35, 49, and 56 d via jugular venipuncture to determine gut development represented by BHB and GL. Results were analyzed using general linear models (SAS v. 9.4, 2024) with backward elimination of independent variables ($P > 0.1$). Independent variables included sex, serum total protein and age. Variables BHB and GL were not different among groups at any time point. Fecal consistency was evaluated daily using a 1–3 scale, with 1 representing normal and 3 indicating watery, loose, or bloody manure. A Kaplan–Meier analysis was used to compare the treatment groups' median days to first diarrhea episode (MedCalc v. 23.1.3). The median time to diarrhea was 14 d and did not differ among groups. Fecal samples were collected at 42 d from 10 calves per treatment to perform metagenomic analysis. Gut community structure and α -diversity did not differ between control and treatment groups. Probiotic feeding did not appear to affect gut or microbiome development under the conditions of this study.

Key Words: calf, probiotic, gut microbiome

2408 Associations between visual body condition score and ultrasound measurements in preweaned Holstein calves. L. N. Laznik*, M. I. Endres, and I. W. Haagen, *University of Minnesota, St. Paul, MN*.

Body condition score (BCS) is used in the dairy industry as a management tool and as a potential indicator of animal welfare; however, these assessments can be highly variable, subjective between assessors, and have not been validated in preweaned dairy animals. The objective of this study was to use ultrasonography to determine the relationships between calf BCS with backfat thickness (BFT), muscle thickness (MT), hip height (HH), and body weight. Preweaned female Holstein dairy calves ($n = 202$) were enrolled and evaluated biweekly for BCS, body weight, and ultrasound parameters. BCS was based upon a scale of 1–3 as used by the FARM Animal Care program. Upon calf arrival and at weaning, HH was measured and heifer mass index (HMI) was calculated; HMI was calculated by dividing calf weight (kg) by HH (m). Ultrasound examinations were conducted in the area between the pins (tuber ischii) and the hooks (tuber coxae) above the thurl on the left side of their body. Ultrasound images were analyzed to determine BFT (mm from the termination of the skin to the termination of the profound fascia) and MT (mm from the termination of the profound fascia to the termination of the gluteal muscle) for each calf. Mixed effects regressions models were used to estimate associations. Regression coefficients for BCS on BFT and MT were 0.150 ± 0.017 and 0.057 ± 0.015 , respectively. The regression coefficient for BCS on weight was 0.216 ± 0.018 . HH and HMI were not associated with BCS. Body condition score is associated with the ultrasound measurements of BFT and MT. This may show that visual scoring can be an appropriate method to evaluate the nutritional status of preweaned dairy calves.

Key Words: body condition score, backfat thickness, ultrasound

2409 Effect of tributyrin and tricaproin in a low-fat milk replacer on feeding behavior and growth in 2-wk-old calves. J. N. Wilms* and L. N. Leal, *Trouw Nutrition R&D, Boxmeer, the Netherlands*.

Milk replacers for calves contain alternative fat sources to milk fat, resulting in lower levels of butyric (C4:0) and caproic acid (C6:0) despite their critical role in development. This study evaluated the effects of incorporating tributyrin (TB) and tricaproin (TC) to a low-fat MR on feeding behavior and growth. Thirty-six Holstein calves (15.5 ± 0.88 d) from 2 dairy farms were blocked based on arrival day and BW. Calves were group-housed with 9 calves per pen, including 3 blocks of pairs and 1 trio. Thus, the block also included the effect of pen. Within each block, calves were randomly assigned to a control MR (CON; $n = 18$) including vegetable fats, or to an MR containing the same fat blend with added TB and TC (TRI; $n = 18$). The TRI MR contained 3.0% (% total FA) C4:0 and 2.3% C6:0. Both MR were isoenergetic with 18% fat, 46% lactose, 23% protein (DM basis). Calves were fed multiple meals of MR (15% solids) through automated feeders able to dispense both MR. From d 0–7, MR allowance increased from 6.0 to 8.0 L/d, remained at 8.0 L/d from d 8–35, and gradually decreased to 0 L/d as a percent per day between d 36–56. Between d 57–70, calves were fully weaned. Starter pellets, chopped straw, and water were provided ad libitum. Body weight was measured weekly. Continuous variables were analyzed using a mixed effects model in SAS with the calf as the experimental unit. The model included treatment, block, and time as a repeated measure, and the interaction between treatment and time. Discrete variables were analyzed using mixed effects logistic regressions. Milk replacer ($P = 0.79$) and starter feed ($P = 0.47$) intake did not differ throughout the study, leading to a similar growth ($P = 0.98$). The number of rewarded visits did not differ ($P = 0.72$); however, in wk 6–7, calves fed TRI tended to have more unrewarded visits ($P = 0.10$). Interrupted MR meals were more frequent in calves fed the CON diet ($P = 0.04$). The number of calves treated for diarrhea ($P = 0.14$) and respiratory diseases ($P = 0.63$) did not differ between treatments. Incorporating TB and TC into MR fed to 2-wk-old calves did not affect feed intake or growth, but influenced feeding behavior, as calves fed TRI appeared to be more eager to drink.

Key Words: dairy calf, butyric acid, caproic acid

2410 Estimating body weight of Holstein and Angus $\times$ Holstein dairy calves using heart girth tape. E. P. Ferdman*¹, H. M. Goetz¹, R. Couto Serrenho¹, J. H. C. Costa³, M. A. Steele², and D. L. Renaud¹, ¹*Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada*, ²*Department of Animal Biosciences, Animal Science and Nutrition, University of Guelph, Guelph, ON, Canada*, ³*Department of Animal and Veterinary Science, College of Agriculture and Life Sciences, University of Vermont, Burlington, VT*.

Determining body weight (BW) of dairy calves is key for evaluating performance and management, but using a digital scale is not always feasible. Weigh tapes offer a practical alternative, yet improvements in genetics and growth rates in recent years may have affected their accuracy. The objective of this diagnostic accuracy study was to validate heart girth tape (Holstein Calf Weigh Tape, Coburg, Whitewater, WI) as a means of estimating the BW of pre-weaned calves compared with a calibrated digital BW scale. From May to August 2024, Holstein ($n = 422$) and Angus $\times$ Holstein ($n = 260$) calves (1 to 11 wk old) were enrolled from calf-raising facilities (Ontario, Canada). At the same time, each calf was weighed once with the digital scale and once with the

weigh tape. The researchers ($n = 6$) assessing the tape measurements were blinded to the digital scale readings. The relationship between the heart girth tape and digital BW scale was analyzed using a mixed linear regression model with a random effect to account for variability between observers, as well as Lin's concordance correlation coefficient (CCC), and Bland–Altman plots. The data were considered precise and accurate when Lin's CCC was very high (>0.90) and the mean bias from the Bland–Altman plot fell within the 95% limits of agreement, including zero. The mean BW was 68.8 ± 18.2 kg, with BW ranging from 28 kg to 143 kg. The BW tape overestimated the digital scale measurement by 1.02 kg ($P < 0.001$, 95% CI: 1.00 to 1.05). The Lin's CCC between BW tape and digital scale measurements was 0.98 ($P < 0.001$, 95% CI: 0.97 to 0.98) for Holstein calves and 0.89 ($P < 0.001$, 95% CI: 0.87 to 0.92) for crossbred calves. The Bland–Altman difference (BW tape – digital scale) was 0.81 ± 4.69 kg (mean $\pm$ SD). These findings indicate that BW tape is a useful tool for estimating pre-weaned calves' BW, though accuracy varies by breed.

Key Words: dairy calf, body weight estimation, surplus calf

2411 Impact of *Bacillus subtilis* 810 and *Bacillus licheniformis* 809 on average daily gain in preweaning calves: A meta-analysis.

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The objective of this meta-analysis was to review all research and field trials evaluating the effects of a probiotic containing *Bacillus subtilis* 810 and *Bacillus licheniformis* 809 on the growth performance of pre-weaned dairy calves. We hypothesized that average daily gain (ADG) would be higher when calves received the 2-*Bacillus*-strain probiotic as compared with control fed calves. After screening against a set of criteria (pre-weaned dairy calves, known concentration of probiotic, individual body weights and feed intakes) 8 studies were included in the meta-analysis (350 calves in total). The meta-analysis was conducted using Excel. The pooled SD was calculated by taking a weighted average of the variances from the 2 groups and adjusted for the sample size. The meta-analysis revealed a significant improvement in ADG among calves receiving the 2-*Bacillus*-strain probiotic compared with controls (81.2 ± 26.9 g/hd per day; $P < 0.01$), supporting the hypothesis that the combination *Bacillus subtilis* 810 and *B. licheniformis* 809 enhances growth performance in pre-weaned dairy calves. This meta-analysis highlights the potential effects probiotics have as a practical, valuable nutritional strategy to support growth during the critical pre-weaning period.

Key Words: calf, probiotic, growth

2412 Key factors influencing growth in dairy calves: A retrospective cohort study.

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The objective of this retrospective cohort study was to explore factors associated with average daily gain (ADG) in dairy calves. Complete calf health records were available from 1,810 female Holstein calves from 8 dairy farms in Ontario, Canada. Electronic scales were used to capture bodyweight (BW) at birth and weaning for each calf. The mean ($\pm$ SD) ADG and weaning age of all calves was 783.5 ± 126.6 g/d and 66.6 ± 8.1 d, respectively. Variables screened in univariable analysis included birth BW, season of birth, time of birth, colostrum feeding volume at first feeding and total in 24 h, colostrum Brix %, colostrum feeding method, serum total protein (STP), weaning age, and incidences of diarrhea, respiratory disease (BRD), navel infection, and inappetence. Season of birth was categorized as winter (December–February), spring (March–May), summer (June–August), and fall (September–November).

Due to lack of linearity, Brix was categorized into quantiles. Variables with $P < 0.20$ in the univariable analysis were included in a multivariable mixed effects linear regression model with farm as a random effect. Manual backward stepwise elimination was used until all variables had $P \leq 0.05$ or were identified as confounders. Spring-born calves (-18.5 g/d; 95% CI: -37.0 to -0.1 ; $P = 0.05$) had lower ADG than winter-born calves. Calves born in the evening had higher ADG than calves born in the morning ($+19.5$ g/d; 95% CI: 5.1 to 33.8 ; $P < 0.001$). Calves fed colostrum with 24%–25% Brix ($+17.3$ g/d; 95% CI: 1.6 to 33.0 ; $P = 0.03$) had greater ADG than calves fed colostrum with $\leq 23\%$ Brix. Further, every 0.1 g/dL increase in STP was associated with a 10.4 g/d increase in ADG (95% CI: 2.6 to 18.3 ; $P < 0.01$). Weaning age was negatively associated with ADG, with each 1-d increase in weaning age resulting in a 2.5 g/day reduction in ADG (95% CI: -3.7 to -1.2 ; $P < 0.001$). Lastly, calves treated for BRD (-62.1 g/d; 95% CI: -75.4 to -48.8 ; $P < 0.001$) or inappetence (-28.0 g/d; 95% CI: -43.3 to -12.8 ; $P < 0.001$) had lower ADG than healthy calves. These results illustrate potentially manageable variables that influence ADG in dairy calves.

Key Words: calf health, growth, morbidity

2413 CalfSim tool: A prediction assessment study of performance prediction of dairy calves.

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CalfSim is a software-based decision support tool designed to assist dairy farmers and consultants in optimizing feeding programs for dairy calves. Although the tool uses the equations from NASEM (2021) as the foundation for estimating energy and protein requirements, as well as starter intake, modifications were made including the use of other solid feed intake prediction and nutrition digestibility and metabolism rate through age. Therefore, the objective of this study was to evaluate the predictions of the CalfSim tool using data from the *Journal of Dairy Science* articles. To assess CalfSim's performance predictions (i.e., body weight), data were extracted from 24 *Journal of Dairy Science* articles published from 2020 to 2025 (since the NASEM 2021 model), along with 3 unpublished studies. Studies were required to report milk allowance, milk or milk replacer composition, starter composition, and body weights at different ages, with ad libitum access to water and starter to be included. Data presented only in graphical form were excluded. In total, 27 studies (76 treatments, 258 body weight observations, and 1,585 calves) were included in the analysis. Model performance metrics for accuracy and precision were calculated using the R programming language, incorporating the random effect of study in the linear regression of predicted and observed body weight. Average BW observed and predicted were 73.3 ± 23.19 kg and 68.6 ± 21.04 kg, respectively. Body weight predictions accounting for study effects demonstrated accuracy ($\beta_0 = 0$ [$P = 0.660$], $\beta_1 = 1$ [$P = 0.097$]) and precision ($R^2 = 0.91$, CCC = 0.93), confirming the tool's high predictive performance. The metrics obtained showed significant study random effects ($\sigma^2_{\text{study}} = 80.80$; $P < 0.05$; ICC = 0.59), highlighted the influence of environment and other farm-specific factors not captured by NASEM (2021) or our adapted model. These findings indicate that CalfSim accurately predicts BW gain under typical conditions, aligning with expected growth patterns across nutritional strategies, though on-farm variability remains a consideration for practical application.

Key Words: calf, decision support tool, modeling

2414 Use of cameras for algorithm generation for the estimation of weight and body measurements of Holstein calves.

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Dairy farming has increasingly incorporated advanced technologies to enhance productivity, animal welfare, and operational efficiency. This study evaluates the reliability of depth cameras for monitoring the weight and body measurements of Holstein calves more efficiently and less stressfully than traditional methods. Seventy-two calves were weighed 4 times preweaning using a mechanical scale. Simultaneously, videos were recorded with an Intel RealSense D455 camera. Videos were processed to extract depth and infrared frames, manually segmented to create masks, and used to train a U-Net model for automated calf body identification. A subsequent model analyzed pixel-based features (e.g., area, volume, eccentricity), generating predictive variables. Finally,

a machine learning regression model estimated body weight, withers height, hip width, and heart girth. Reference measurements used for validation included manual methods: an anthropometric ruler for withers height and hip width, a measuring tape for heart girth, and a mechanical scale (ICS-300, Coimma Ltda, Brazil) for weight. Pearson correlation and linear regression assessed prediction accuracy, showing high correlations (body weight: $r = 0.93$, $R^2 = 0.86$; withers height: $r = 0.86$, $R^2 = 0.74$; hip width: $r = 0.84$, $R^2 = 0.70$; heart girth: $r = 0.87$, $R^2 = 0.74$). Results confirm the viability of this technology as a precise, automated alternative. Automated monitoring reduces labor, minimizes animal stress, and enhances data accuracy, improving decision making and farm productivity. This approach advances dairy farming by optimizing herd management through precision livestock techniques.

Key Words: weight estimation, calf, automation

Physiology and Endocrinology 2: Posters

2415 Metabolic and endocrine responses to different carbohydrates as substitute for lactose in milk-fed calves. J. J. Gross*, G. Bierlein, and R. M. Bruckmaier, *Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

We investigated which effects different carbohydrate sources in milk exerted on metabolism of preweaning calves. Male and female calves ($n = 38$, age: 16.0 ± 0.3 d, BW: 53.7 ± 1.0 kg) were assigned to 5 experimental diets (4 kg per meal, 2 meals/d) fed for 3 consecutive days. Different carbohydrates were added to a milk replacer: Lactose (LAC), glucose (GLU), galactose (GAL), glucose + galactose (G/G), and xylose (XYL). Blood samples were taken from the jugular vein on the last day of treatments, right before feeding in the morning (T1), 2 (T2) and 7 (T3) hours later. Concentrations of glucose, nonesterified fatty acids (NEFA), β -hydroxybutyrate (BHB), triglycerides (TG), insulin, and glucagon were determined in plasma. Data were evaluated using a mixed model (SAS, v9.4) with treatment, time point of sampling, and treatment $\times$ time interaction as fixed effects and the individual calf as repeated subject. Plasma glucose concentration increased after feeding from T1 to T2 in GLU ($P < 0.05$), but not in calves fed LAC, GAL, G/G, and XYL. Nonesterified fatty acid concentrations were increased by trend in GAL at T2 ($P = 0.06$). Plasma BHB concentrations increased ($P < 0.05$) in all groups from T1 to T2. Blood TG concentrations were increased in all groups at T2 compared with T1 (GLU, GAL, XYL: $P < 0.05$; LAC: $P = 0.08$; G/G: $P = 0.09$). Whereas insulin concentrations increased after feeding from T1 to T2 (G/G: $P < 0.05$; GLU: $P = 0.09$), glucagon concentrations were greater at T2 (XYL: $P < 0.05$; GLU: $P = 0.08$) and T3 (GLU, G/G, XYL, GAL: $P < 0.05$; LAC: $P = 0.06$) compared with T1. Despite a similar gross composition of the experimental diets, digestion, absorption, and postabsorptive metabolism seem to differ between the carbohydrate supplements. In calves receiving XYL, a more catabolic status could be shown as XYL is absorbed but cannot be used in intermediary metabolism.

Key Words: milk replacer, lactose, carbohydrate

2416 Effects of acepromazine maleate on glucose metabolism in 12-week-old dairy heifers. S. J. Blair*, C. C. Williams, M. C. Orantes, and E. L. Oberhaus, *Louisiana State University, Baton Rouge, LA.*

Acepromazine maleate (ACE) is a phenothiazine tranquilizer commonly used in veterinary medicine, but its effects on glucose metabolism in dairy calves when used as a form of chemical restraint during metabolic research procedures remain unclear. Two experiments were conducted, each in a crossover design, where 10 Holstein heifers between 12 and 15 weeks of age were pretreated with either ACE (0.02 mg/kg BW, IV) or saline control 30 min before either intravenous insulin tolerance test (ITT) or intravenous glucose tolerance test (IVGTT). All treatments and sampling occurred via jugular catheter. In the first study, an ITT was conducted in which calves received an IV bolus of insulin (75 mIU/kg BW) followed by serial blood sampling -10, 0, 20, 30, 40, 50, and 60 min relative to insulin infusion. Calves underwent both treatments (ACE and control) with a 24 h washout period. Blood glucose concentrations were immediately measured using the OneTouch Ultra mini hand-held glucometer. Blood glucose concentrations were analyzed via repeated measures ANOVA on log-transformed dose response data to determine dose significance at individual time points. Results indicate that ACE administration did not alter the glucose response to insulin, as ACE-treated calves exhibited similar glucose clearance in treatments and

controls in response to ITT. In study 2, an IVGTT was conducted in which a glucose bolus (0.5 g/kg BW, IV) was administered, and blood samples were collected -30, -20, 0, 5, 10, 15, 20, 25, 30, and 35 min relative to glucose infusion to assess the insulin response. A 7-d washout period was allowed between ACE and control treatments. Insulin over time was compared between groups using a mixed effects ANOVA. Total area under the curve and area of positive peaks were compared via a Student's t -test using GraphPad Prism. No statistical differences were found between groups. These findings suggest that ACE may be used as an acceptable form of chemical restraint during either ITT or IVGTT without significant interference in the response to treatment.

Key Words: acepromazine, glucose metabolism, insulin challenge

2417 Milk-derived peptide isoleucine-proline-proline supplementation altered glucose, lipid, and amino acid metabolism in primary bovine hepatocytes. E. Kolodziej*, J. R. Daddam, G. Ahmad, M. Sura, and Z. Zhou, *Michigan State University, East Lansing, MI.*

Milk-derived bioactive peptides have been extensively studied for their metabolic regulatory effects in humans and rodents, yet their impact on cows remains largely unexplored. During early lactation, hepatocytes in the bovine liver experience metabolic stress as they become overwhelmed by the influx of fatty acids mobilized from adipose tissue. The objective of this study was to assess the impact of isoleucine-proline-proline (IPP), a milk-derived peptide, on primary bovine hepatocytes exposed to fatty acids. control (CON): customized medium with amino acids, volatile fatty acids, fatty acids (FA), glucose, choline, insulin, and albumin concentrations equal to circulating levels in cows 4d postpartum; or IPP: CON + 100 μ M IPP. After 72 h of culture, hepatocytes were harvested and RNA was extracted for RNA sequencing. Raw reads were trimmed (Trimmometry), aligned to the bovine reference genome (STAR), and assembled and quantified (Salmon). The differential gene expression (DEG) analysis was performed with DESeq2 (R environment). Genes with adjusted P -value (FDR) < 0.05 were considered differentially expressed genes (DEGs). Functional enrichment analysis of the DEGs were carried out using the ingenuity pathway analysis (IPA) with Z-score of 2.0 as the cutoff for significant pathways. A total of 18,153 transcripts corresponding to 5,232 genes were identified, among which 469 were unique DEGs (FDR < 0.05). Enrichment analysis revealed that Glucose Metabolism, Peroxisomal Lipid Metabolism, Branched-Chain Amino Acid Catabolism, and Fatty Acid β -oxidation are top-enriched pathways in IPP supplemented hepatocytes compared with CON. These results indicate that IPP supplementation altered hepatic glucose, lipid, and amino acid metabolism. Quantification of genes and proteins within these pathways is necessary to determine the specific impact of these metabolic changes.

Key Words: isoleucine-proline-proline (IPP), transcriptomics, metabolism

2418 Effect of balancing metabolizable His alone or with Met, Lys, and Leu on plasma and urine metabolites. M. Killerby*, K. Ruh¹, L. C. Ribeiro¹, V. Pszczolkowski¹, A. Larsen¹, E. Cohan¹, A. Benn¹, A. Hoppmann¹, D. Sherlock², and S. Arriola Apelo¹, ¹*Animal and Dairy Science, University of Wisconsin-Madison, Madison, WI*, ²*Adisseo USA Inc., Alpharetta, GA.*

We aimed to investigate the metabolism of lactating cows fed diets balanced for His and/or Met, Lys, and Leu, that showed increased milk components yield. Thirty-two Holstein cows (77 ± 17 DIM) were enrolled in a replicated 4×4 Latin square study with 28-d periods. The 4 dietary treatments were arranged in a 2×2 factorial with low (56 g/d; LH) or high (69; HH) metabolizable His (H), and low ($60 + 192 + 230$; LMKL) or high ($77 + 238 + 257$; HMKL) metabolizable Met + Lys + Leu (MKL) balanced by using varying amounts of blood meal, corn gluten meal, soyhulls, and rumen-protected Met+Lys. Coccygeal blood ($\times 10$) and urine ($\times 8$) samples were collected on d 25–26 of each period, staggered to represent samplings every 2 and 3 h between milkings in a day, respectively. Data were analyzed with a mixed linear model that included the fixed effects of H, MKL, HxMKL interaction, period, and square, and the random effect of cow within square. Compared with LH, HH increased plasma concentrations of Asp by $0.5 \mu\text{M}$ ($P = 0.05$), His by $42.4 \mu\text{M}$ ($P < 0.001$), and Val by $58 \mu\text{M}$ ($P < 0.001$), but decreased Tyr $-15.5 \mu\text{M}$ ($P = 0.001$). Plasma Met ($P < 0.001$) was increased and Lys ($P = 0.07$) tended to increase by HMKL vs. LMKL (35.5 vs. 26.2, and 102 vs. 92 μM , respectively) but no effect was observed on Leu ($P = 0.13$). Total essential AA in plasma were increased by HH vs. LH (1,062 vs. 947 μM ; $P = 0.04$) whereas neither factor affected NEAA or total AA ($P > 0.1$). Plasma urea N increased with HMKL (17.5 mg/dL) vs. LMKL (16.9; $P < 0.001$). Plasma glucose was decreased by H and MKL (-2.7 mg/dL; -2.6 ; $P \leq 0.02$), whereas insulin was not affected by either factor ($P > 0.1$). Urinary output of allantoin increased with HH vs. LH (565 vs. 520 mmol/d; $P < 0.001$), whereas it decreased with HMKL vs. LMKL (525 vs. 560; $P = 0.006$), resulting in higher estimated microbial N flow with HH ($+43$ g N/d; $P < 0.001$) and lower with HMKL (-23 g N/d; $P = 0.006$). The increase in plasma Met, Lys, and urea N, and previously reported increase in MUN and urine urea N suggest that although MKL cows increased milk protein yield, postabsorptive AA efficiency diminished with AA supplementation.

Key Words: histidine, amino acids, metabolites

2419 Effect of oral bolus of calcium and vitamin D glycoside on blood calcium concentration during early lactation. L. A. Alvarado Rojas^{*1}, R. A. Villalobos Agüero², J. P. Goff³, and J. A. Arce Cordero¹, ¹Department of Animal Sciences, University of Costa Rica, San José, Costa Rica, ²Animal Nutrition Research Center, University of Costa Rica, San José, Costa Rica, ³Accunutrition, Ames, IA.

Oral Ca boluses at calving and vitamin D analogs have been used separately to prevent hypocalcemia with limited success. Our objective was to evaluate the effects of oral supplementation at calving with a bolus containing Ca and vitamin D glycosides from *Solanum glaucophyllum* on blood Ca of cows in early lactation. Sixty-four multiparous Jersey cows were used in a randomized complete block design with 32 blocks and cows were blocked by 305 d milk production in the previous lactation. Treatments were randomized within each block and consisted of (1) control without bolus (CON), and (2) supplementation with 2 boluses at the time of calving; each bolus providing 39 g of Ca as Ca chloride and Ca propionate and 5 g *Solanum glaucophyllum* dried leaf material (BOL). Blood samples were collected daily from each cow before morning feeding on d 0 to 7 postpartum and centrifuged at $1,000 \times g$ for 15 min. Serum samples were analyzed for Ca, Mg, P, and glucose following methods of Arsenazo III, Xylidyl Blue, phosphomolybdate, and GOD-POD, respectively. Blood β -hydroxybutyrate (BHB) was measured on d 7 and 28 postpartum with a hand-held meter (OptiumX-

ceed), body condition score (BCS) estimated on d 0, 14, and 28, and daily milk production recorded from d 0 to d 28. Data were analyzed with SAS 9.4 as repeated measures, and the model included the fixed effects of treatment (T), day relative to calving (D), and their interaction ($T \times D$) and random effects of block, cow (T), and lactation. Blood Ca (± 0.24 SEM) was greater in BOL than CON ($T \times D$, $P < 0.01$) on d 1 (8.0 and 6.5 mg/dL) and d 2 (8.3 and 7.0 mg/dL), respectively. Blood P (± 0.41 SEM) was greater in BOL than CON ($T \times D$, $P < 0.01$) on d 2 (7.2 and 5.5 mg/dL), d 3 (8.5 and 6.3 mg/dL), and d 4 (8.7 and 6.6 mg/dL), respectively. Blood Mg (± 0.10 SEM) was lower in BOL than CON ($T \times D$, $P < 0.01$) on d 1 (2.3 and 3.0 mg/dL) and d 2 (2.2 and 2.7 mg/dL), respectively. Milk production, BCS, blood glucose, and BHB changed over time (D , $P < 0.05$) but were similar between treatments. Supplementing a bolus with Ca and vitamin D glycoside at calving temporarily increased blood Ca and P in early postpartum.

Key Words: hypocalcemia, mineral

2420 Plasma fatty acids differ between mid-lactation cows with low vs. high residual feed intake. M. Paiva Rodrigues¹, S. J. Kendall¹, M. J. Caputo^{1,2}, K. M. Kennedy¹, M. J. VandeHaar³, Z. Zhou³, F. Peñagaricano¹, K. A. Weigel¹, and H. M. White^{*1}, ¹University of Wisconsin–Madison, Madison, WI, ²Council for Dairy Cattle Breeding, Bowie, MD, ³Michigan State University, East Lansing, MI.

Improving feed efficiency (FE) requires phenotypic energy intake and output quantification in a large reference population. Exploration of fatty acids (FA) that may differ between high- and low-FE cows may provide an avenue for less intensive identification of FE phenotypes. The objective of this study was to determine the plasma FA profiles of high and low-FE cows from 2 separate studies and identify potential biomarkers for FE. To identify high and low-FE cows, residual feed intake (RFI) from multiparous Holstein dairy cows was calculated using DMI, metabolic BW, change in BW, trial, and ECM. Blood samples were collected simultaneously from the mammary vein and tail vessel from the 31 highest and 32 lowest RFI cows ($n = 63$) across the 2 studies. Plasma samples were methylated via Folch extraction, spiked with C19:0 as the internal standard and chloroform extracted, after which 14 FA were quantified via gas chromatography/mass spectrometry. Data were analyzed using PROC MIXED (SAS 9.4) with fixed effect of RFI and random effect of sampling day and trial. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. Results are reported as means [95% CI]. Tail vessel FA analysis revealed differences ($P \leq 0.04$) between low-RFI (high FE) compared with high-RFI (low FE) cows for C16:1 (1.20 [1.07, 1.34] vs. 0.97 [0.83, 1.10]), C18:0 (20.7 [17.80, 23.60] vs. 18.55 [15.70, 21.40]), and C22:0 (0.44 [0.36, 0.52] vs. 0.38 [0.30, 0.46]). A tendency ($P \leq 0.07$) was detected for C16:0 (14.58 [12.53, 16.63] vs. 13.36 [11.34, 15.38]) and C17:0 (1.02 [0.84, 1.19] vs. 0.92 [0.75, 1.10]). Differences ($P \leq 0.01$) for mammary vein included C16:1 (1.07 [1.00, 1.15] vs. 1.00 [0.98, 1.08]) and C18:1cis (7.78 [6.44, 9.12] vs. 6.71 [5.39, 8.03]). These differences in plasma FA suggest variations in FA utilization and metabolism between RFI group between high- and low-FE cows. These differences may suggest differences in fatty acid oxidation between high and low-FE cows. Importantly, either alone or combined with other variables, FA that differ by FE status could potentially serve as markers for FE to differentiate efficient and inefficient lactating dairy cows.

Key Words: feed efficiency, biomarker

Production, Management, and the Environment 2: Posters

2421 Associations of the Fresh Cow Index with performance later in lactation. A. De Vries*¹ and R. H. Fourdraine², ¹University of Florida, Gainesville, FL, ²Dairy Records Management Systems, North Carolina State University, Raleigh, NC.

The Fresh Cow Index (FCI) is the ratio of actual energy-corrected first test-day (TD) milk yield (ECM) and predicted ECM $\times$ 100. An FCI significantly less than 100 may indicate that the cow had a poor transition into lactation. The objective of this study was to associate the FCI with milk production and herd removal (culling) later in lactation. For FCI model building, data collected between 2020 and 2024 were provided by Dairy Records Management Systems and included 989,296 first parity records from 1,938 herds and 2,049,356 older parity records from 3,094 herds. Linear mixed models were developed to predict ECM at first TD (5 to 40 DIM) for first-parity and older parity cows. The models included variables related to year, calving age, genetic traits, dam performance, season, region, milking frequency, breed, abortion, calving ease, gestation length, and DIM at first TD. The older parity model also included variables related to previous parity dry period length and days open. Herd was included as a random effect. The coefficients from the regression models were then used to predict ECM at first TD. To determine the associations of low versus high FCI with nonvoluntary removal rates and milk production, data from 409,384 first and 1,252,150 older parities were grouped by 10 FCI increments from FCI <70 to FCI $\geq$ 130. Removal rates for first-parity cows decreased from 26.7% for cows with FCI <70% to 23.2% for cows with FCI $\geq$ 130, whereas peak milk yield increased from 31 kg to 43 kg. Similarly, 305-d ECM increased from 9,297 to 12,293 kg for FCI <70 to FCI $\geq$ 130. For older cows, removal rates decreased from 37.7% to 33.1%, peak milk yield increased from 41 kg to 59 kg, and 305-d ECM increased from 10,596 kg to 14,528 kg, respectively. Across parities, cows with FCI <100 were less likely to start a subsequent lactation. Cows with FCI < 80 were predominantly removed for production reasons and cows with FCI >80, if removed, were increasingly removed for reproduction reasons. In conclusion, monitoring FCI grouped by calving cohort will provide the dairy producer with a tool to evaluate transition cow management.

Key Words: transition, monitor, management

2422 Lactating dairy cow behavior under commercial application of “smart” versus a conventional cooling system. I. M. Toledo*, H. Olmo, and G. E. Dahl, University of Florida, Gainesville, FL.

Active cooling systems are widely used to reduce the negative effects of heat stress and optimize dairy cow performance during all phases of the lactation cycle. Shade, ventilation and soaker systems are extensively used in dairy farms as cooling strategies. The objective of this study is to evaluate the behavior of lactating dairy cows under an automated “smart” system (AgPro, Paris, TX) for control of soaker output relative to a conventional approach that relies on set timing after reaching a specified temperature threshold. We hypothesize that the smart system will reduce water usage compared with the conventional system while not affecting the behavior of lactating dairy cows. Automated monitoring devices (Nedap, Groenlo, the Netherlands) were used to assess behavioral activity of lactating cows under an automated smart system (SS; n = 12) or conventional cooling systems (CON; n = 12). Cows received a leg tag to measure daily lying time, number of steps, and standing bouts, and a neck tag to measure eating and rumination time. Behavioral activities were recorded for 17 d, during which the

average temperature-humidity index was 75. All cows were housed in a sand-bedded freestall barn equipped with a cooling system of shade, fans and soakers. Soaker systems water usage (WU; L/d) per pen were recorded daily. As expected, no differences in behavioral activities were observed between the groups. Eating (232.6 vs. 241.0 $\pm$ 21 min/d; P = 0.78), rumination (241.3 vs. 250.1 $\pm$ 8.2 min/d; P = 0.41), lying (738.9 vs. 714.0 $\pm$ 34 min/d; P = 0.61) and standing (701.0 vs. 726.0 $\pm$ 34 min/d; P = 0.61) did not differ between SS and CON. Steps (5,133 vs. 4,718 $\pm$ 185 steps/d; P = 0.13), stand bouts (11 vs. 13 $\pm$ 0.8 stands/d; P = 0.30) and inactivity (580.1 vs. 545.7 $\pm$ 26 min/d; P = 0.37) were also similar between SS and CON. Water usage was significantly higher in the CON pen compared with SS (34.2 vs. 19.6 $\pm$ 1.8, L/day $\times$ 1,000; P < 0.001). These results suggest that the automated smart system is as effective as a conventional soaker system to actively cool cows and does not affect behavior while using significantly less water.

Key Words: water use, active cooling, smart system

2423 Evaluating the RuFaS model: Insights into NH₃ emission simulations via data assimilation. H. Hu*¹, H. Xu², Y. Luo², and K. F. Reed³, ¹Department of Animal Science, Cornell University, Ithaca, NY, ²School of Integrative Plant Science, Cornell University, Ithaca, NY, ³USDA-Dairy Forage Research Center, Madison, WI.

Ammonia (NH₃) poses significant risks to the environment and human health, serves as a precursor to nitrous oxide, and represents a source of nutrient loss and inefficiency. Quantification of NH₃ emissions on farms is challenging because manure management practices and environmental conditions can vary widely. Process-based modeling offers a more robust approach than empirical methods for addressing this complexity. The Ruminant Farm Systems (RuFaS) model combines process-based models across the farm system to estimate whole-farm NH₃ emissions. In particular, an established NH₃ volatilization equation is used to assess emissions from the barn floor. This study develops a data assimilation algorithm to estimate farm-specific parameters for NH₃ emissions. To enhance computational efficiency, we isolated the NH₃ emissions method from the RuFaS model and conducted a first-order sensitivity analysis to identify key parameters for estimation. Among the parameters analyzed (manure pH, housing-specific constant, barn area, and manure density), manure pH had the highest sensitivity index (0.661), indicating its strong influence on NH₃ emissions. Using this insight, we developed a Metropolis–Hastings algorithm to estimate manure pH and evaluated the method with simulated housing NH₃ emissions data. The proposed data assimilation method consistently converged to the target pH value of 7.7, regardless of the initial values (6.5, 7.7, or 8.5), with an average acceptance rate of 21.5%. These results demonstrate the algorithm’s potential for accurately estimating farm-specific parameters. Future work will focus on extending the algorithm to estimate multiple parameters simultaneously and validating it with on-farm NH₃ measurements. This work presents an opportunity to advance the development of regional NH₃ emission inventories tailored to specific manure management practices.

Key Words: manure ammonia, data assimilation, Ruminant Farm Systems (RuFaS)

2424 Effect of whole lactation chromium propionate supplementation in automated milking systems on body weight, body condition score, production, and reproduction. T. A. Westhoff¹,

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Supplementing diets with chromium has been shown to improve tissue's sensitivity to insulin, but long-term effects of chromium supplementation on BW and BCS remain unknown. The objective was to determine whether chromium propionate (Cr) supplementation during lactation would alter BW, BCS, milk yield, milk composition, reproductive success, and herd removal. Holstein cows (parity = 2.4 ± 0.9 ; 6.7 ± 3.1 DIM), blocked by calving date and balanced by pen, were randomly assigned in an automated milking system (AMS) to either a concentrate pellet (Con; $n = 95$) or the same pellet formulated with Cr at 0.487% of DM (Cr; KemTRACE Chromium 0.04%, Kemin Industries Inc., Des Moines, IA; $n = 96$). Treatments (trt) were fed until 308 DIM. Milk yield, milk composition indications, BW, herd removal, and reproduction records were collected from the AMS software. Body condition score was determined once monthly. Data were analyzed using mixed effects repeated measures ANOVA models with the effects of trt, time, parity, pen, and trt $\times$ time. Herd removal and time to pregnancy were analyzed with Cox proportional hazard models with the effects of trt and parity. Body weight (687 vs. 685 ± 6 kg; $P = 0.75$) and BCS (3.31 vs. 3.30 ± 0.04 ; $P = 0.69$) did not differ between Cr and Con, respectively. Feeding Cr did not affect milk yield (44.3 vs. 44.2 ± 0.9 kg/d; $P = 0.93$), fat % (4.03% vs. $3.98\% \pm 0.05\%$; $P = 0.38$), protein % (3.16% vs. $3.16\% \pm 0.02\%$; $P = 0.92$), or ECM (48.1 vs. 47.5 ± 0.9 kg/d; $P = 0.62$) compared with Con, respectively. Hazard ratio for time to pregnancy [0.94 (0.68 – 1.30)] and herd removal [1.62 (0.93 – 2.82)] did not differ by trt. Overall, Cr supplementation throughout lactation did not increase BW and BCS nor did it affect productive or reproductive parameters.

Key Words: Chromium propionate, body weight, BCS

2426 Effect of litter size, parity, and month of lambing on lactation milk yield and fat percentage in Turkish Awassi sheep. P. Usta* and S. Dikmen, *Department of Animal Science, Faculty of Veterinary Medicine, Bursa Uludag University, Bursa, Turkey.*

Awassi sheep, a prominent breed originating from the Middle East, are well-regarded for their adaptability to arid environments and their robust production traits under traditional conditions. This study was

conducted to investigate the lactation performance of Turkish Awassi sheep, focusing on average milk yield and fat percentage across different months of lambing, litter sizes, and parities. A total of 1,718 test-day records from 198 lactations of 82 Awassi sheep, which were reared over 3 consecutive years, were used for this study. In the study, the average litter size, gestation period, age, fat percentage, lactation milk yield, and lactation period were determined as 1.5 ± 0.01 ; 150 ± 0.3 d; 2.8 ± 0.05 years; $6.1\% \pm 0.07\%$; 125 ± 3.4 L; and 159 ± 2.2 d, respectively. The average test-day milk yield increased with the application of better management conditions between the first and third years: 579 ± 53.7 mL, 944 ± 50.4 mL, and 979 ± 49.6 mL ($P < 0.001$), respectively. Ewes lambing in February had a higher average lactation milk yield (153.2 ± 11.4 L) compared with those lambing in March (132.5 ± 14.3 L; $P < 0.05$). Litter size significantly affected both lactation milk yield and test-day milk yield, with triplet-bearing ewes yielding the most (189.2 ± 33.1 L; $P < 0.01$) compared with single-lambing ewes (109.3 ± 6.0 L). The effect of parity was also significant, as ewes in their third lactation tended to produce more test-day milk yield (957 ± 49.6 mL) compared with first-parity ewes (730 ± 61.6 mL; $P < 0.001$). As a result, the month of lambing had a significant effect on milk yield, and improved management conditions positively affected milk production in Turkish Awassi sheep.

Key Words: Turkish Awassi, milk yield, litter size

2427 Comparison of milk yield, composition, and fatty acid profile from morning and evening samples in Holstein cows at different milking frequencies. L. C. Copetti^{1,2}, J. H. Carneiro², A. M. Fillus³, L. B. Los⁴, D. A. Garrett Neto⁵, A. A. Valloto⁶, J. A. Horst⁶, and R. Almeida^{*1}, ¹Universidade Federal do Paraná, Curitiba, Paraná, Brazil, ²Dairy Inside Consultoria, Castro, Paraná, Brazil, ³Castrolanda Cooperativa Agroindustrial, Castro, Paraná, Brazil, ⁴Frísia Cooperativa Agroindustrial, Carambei, Paraná, Brazil, ⁵Capal Cooperativa Agroindustrial, Arapoti, Paraná, Brazil, ⁶Associação Paranaense dos Criadores de Bovinos da Raça Holandesa, Curitiba, Paraná, Brazil.

This study aims to investigate intraday variations in milk yield, composition and fatty acid (FA) profile in Holstein cows at different daily milking frequencies. The study covered 20 commercial herds (10 at $2\times/d$, and 10 at $3\times/d$ milkings). Milk yield and milk samples were collected from 6,594 cows from both groups ($2\times/d$ and $3\times/d$) at each milking within

Table 1 (Abstr. 2427).

Variable	Milking $2\times/d$ ($n = 2,416$ cows)			Milking $3\times/d$ ($n = 4,178$ cows)			
	First milking	Second milking	SEM	First milking	Second milking	Third milking	SEM
Milk yield (kg)	20.87 ^a	20.42 ^b	0.154	15.97 ^a	14.69 ^b	14.54 ^c	0.105
Fat (%)	3.85 ^b	4.01 ^a	0.026	3.21 ^c	3.40 ^a	3.31 ^b	0.021
Total protein (%)	3.32	3.34	0.012	3.33 ^b	3.34 ^b	3.37 ^a	0.008
Lactose (%)	4.46	4.47	0.006	4.55	4.56	4.56	0.004
Total solids (%)	12.57 ^b	12.77 ^a	0.035	12.06 ^b	12.29 ^a	12.21 ^a	0.026
MUN (mg/dL)	13.42 ^b	13.88 ^a	0.101	10.98 ^a	11.05 ^a	10.51 ^b	0.082
De novo FA (g/100 g milk)	0.91 ^b	0.93 ^a	0.007	0.74 ^b	0.77 ^a	0.75 ^b	0.007
Mixed FA (g/100 g milk)	1.19 ^b	1.26 ^a	0.008	1.01 ^c	1.06 ^a	1.03 ^b	0.006
Preformed FA (g/100 g milk)	1.22 ^b	1.29 ^a	0.008	1.03 ^c	1.13 ^a	1.09 ^b	0.006
De novo FA (g/100 g FA)	27.29 ^a	26.65 ^b	0.056	26.50 ^a	25.88 ^c	26.14 ^b	0.053
Mixed FA (g/100 g FA)	35.84 ^b	36.29 ^a	0.046	36.18 ^a	35.86 ^b	35.91 ^b	0.039
Preformed FA (g/100 g FA)	36.80 ^b	37.04 ^a	0.076	37.41 ^c	38.36 ^a	38.07 ^b	0.073

^{a-c}Values in a row with different superscripts differ ($P < 0.01$).

a 24-h period, from September to December 2024. Milk composition was analyzed using Fourier-transformed mid-infrared (FTIR) spectroscopy. Data were analyzed using the MIXED procedure of SAS. Milk yield, composition, and FA profile were compared between milking time within farm for each group. The statistical model included herd and milking time (first and second for 2×/d; first, second, and third for 3×/d, respectively) as fixed effects, and cow as a random effect. In 2×/d cows, the first milking (morning) at 2×/d showed higher ($P < 0.01$) milk yield and de novo FA (g/100 g FA), and lower ($P < 0.01$) milk fat, total solids, MUN, mixed FA, and preformed FA (g/100 g FA) than the evening milking. In a similar manner, the first milking at 3×/d showed higher ($P < 0.01$) milk yield and de novo FA (g/100 g FA), and lower ($P < 0.01$) milk fat, total protein, total solids, and preformed FA (g/100 g FA) than the second and third milkings (Table 1).

Key Words: mid-infrared spectroscopy, milking

2428 Zootechnical indexes: A comparative study between traditional DHI herds and new DHI herds. M. M. Marquetti¹, G. A. Z. Bueno¹, D. R. S. Oliveira², J. A. Horst³, A. A. Valloto³, L. T. Dias¹, and R. Almeida^{*1}, ¹Universidade Federal do Paraná, Curitiba, Paraná, Brazil, ²Castrolanda Cooperativa Agroindustrial, Castro, Paraná, Brazil, ³Associação Paranaense de Criadores de Bovinos da Raça Holandesa, Curitiba, Paraná, Brazil.

This study aimed to compare indexes from large and traditional herds in the DHI (Dairy Herd Improvement) service with those observed in small and medium-sized growing herds, which began DHI monitoring only in July 2023. DHI reports ($n = 3,029$) were compiled containing information on the zootechnical indexes of 277 herds (97 traditional and 180 new herds), located at Castro county, Paraná State, Southern Brazil, from July 2023 to December 2024. Statistical analysis was performed using the MIXED procedure of the SAS, and herd category (new or traditional), breed and season were considered as fixed effects, and herd as a random effect. New herds had an average 88 lactating and dry cows, whereas traditional herds, 373 cows. Daily milk yield was higher for traditional DHI herds than for new DHI herds (30.76 vs. 20.97 kg/d; $P < 0.01$). Traditional DHI herds had a tendency to have a lower DIM (222.9 vs. 240.1 d; $P < 0.05$) than new DHI herds, although both are above the benchmark of 180 DIM. No differences were found between traditional DHI and new DHI herds for milk fat content (3.97 vs. 4.05%; $P = 0.14$), milk total protein content (3.44 vs. 3.43%; $P = 0.79$), and milk total solids (12.97 vs. 12.96%; $P = 0.87$), suggesting that the payment system for milk components benefits both large and traditional DHI herds as well as small and medium new DHI herds. The production of fat plus true protein was higher for traditional herds than new herds (2.18 vs. 1.52 kg/d, $P < 0.01$). Traditional DHI herds showed a higher lactose content (4.57 vs. 4.47%; $P < 0.01$) and a lower somatic cell count (271.73 vs. 346.21×10^3 cells/mL; $P < 0.01$) than new DHI herds, indicating higher milk quality on traditional DHI herds. Cow average age was lower for traditional DHI herds (51.87 vs. 63.11 mo; $P < 0.01$), suggesting traditional DHI herds cull more cows. No differences were found for calving interval (439.6 vs. 444.7 d; $P = 0.46$) and days open (157.1 vs. 163.5 d; $P = 0.36$) for traditional and new DHI herds, respectively. In conclusion, our results demonstrate that measuring zootechnical indexes using monthly milk recording can contribute to a faster progress of dairy herds.

Key Words: dairy herd improvement, herd size, milk recording

2429 An automated tool for individual cow and group daily cash flow estimation for improved decision making in dairy farms.

A. Geroge*, M. M. Perez, and J. O. Giordano, *Cornell University, Ithaca, NY.*

The objective was to create a tool that automatically calculates in real-time cash flow (CF) for individual cows and groups. Every day the tool integrates in a database individual cow daily milk yield and components (AfiLab) and body weight (BW) collected (Afiweigh) after milking. Calving, health, reproduction, preventive interventions, sale, and death events are also retrieved from the herd management software (Dairy-Comp305) and added to the database. Total daily CF is the aggregation of milk and calf revenue, and feed, health, reproductive, and replacement costs. Milk revenue is the multiplication of daily milk fat and protein yield by component market prices. Feed costs are the multiplication of predicted dry matter intake estimated with the NRC equation ($DMI = 0.372 \times 4\% \text{ FCM} + 0.0968 \times BW^{0.75}$) by feed costs per kilogram of feed. Health and preventive interventions (e.g., vaccinations) costs are the accumulation of individual treatments or interventions recorded for each health disorder or preventive intervention event. Reproductive costs for each event are the aggregation of semen costs, hormonal treatments, labor and supplies for insemination, and pregnancy testing. Detection of estrus is applied as a daily cost. Calf revenue is the value of a calf based on current market values depending on sex and breed. Replacement costs are the aggregation of beef salvage value, the replacement cost, and the value of the calf from the replacement. To illustrate the tool, the difference in CF to 60 DIM was estimated for cows with mastitis ($n = 17$), metritis ($n = 11$), or digestive disorders ($n = 22$) matched with the same number of healthy cows controlling for parity, calving date, net merit, and previous lactation yield. Cows with metritis, mastitis, and digestive disorders had \$98, \$125, and \$109 less CF, respectively, than healthy cows. In summary, a software tool that automatically integrates multiple sources of performance and events data from individual cows can calculate overall daily CF including major sources or revenues and expenses. This tool could facilitate improved economic decision making at the cow and herd level.

Key Words: profitability, dairy cow, automated

2430 Effect of the technological level in the profitability of milk production system from “Balde Cheio” project in the Leste Rondoniense mesoregion. L. Freiria¹, E. M. Reis^{*1}, F. L. Dantas¹, M. A. Lopes², and G. A. Almeida Junior³, ¹Federal University of Acre, Rio Branco, Acre, Brazil, ²Federal University of Lavras, Lavras, Minas Gerais, Brazil, ³Federal University of Espirito Santo, Alegre, Espirito Santo, Brazil.

We analyzed the effect of the technological level on the rentability of 14 demonstrative units (DU) from the “Balde Cheio” project, in the Leste Rondoniense mesoregion, from January to December 2020. It was also intended to identify the components that exert greater representation of total costs (fixed and variable) and operating costs (effective and total). Statistical analysis was initially conducted with the Shapiro–Wilk test assessed the normality of the samples, and Levene’s test evaluated the equality of variances among the continuous variables. For dependent variables that followed a normal distribution, ANOVA was employed to compare the different technological strata. The results were expressed as means and standard deviations, providing a clear and standardized presentation of the data. The threshold for statistical significance was set at $P < 0.05$. The 14 DUs were segmented into 3 technological levels: low, medium, and high. Units with advanced features, such as mechanical milking systems and significant investments in modern machinery and facilities, were categorized as high-level, encompassing 4 units. In contrast, 3 units were categorized as low-level, characterized by manual milking processes and limited investment in machinery or

infrastructure. The remaining 7 units, which did not fit strictly into the high or low categories, were designated as medium-level. It was found that the technological level did not influence the profitability and rentability of the strata. The medium and high technology strata showed a positive result, obtained by the collaboration of external factors such as the price of milk and arroba in the analyzed period, however technological development was essential for achieving long-term capitalization for the high technology stratum; the low technology stratum had a positive net margin, thus fixed costs were not covered for this stratum. The items that make up the effective operating cost that were most representative for all strata were food, followed by labor and energy, in descending order; the component items of the total cost with greater representation were food (averages of 31.54%, 38.22%, and 39.31% for the low, medium, and high technological level strata, respectively), land remuneration (averages of 24.01%, 8.69%, and 11.45% for the low, medium, and high technological level strata, respectively) and of the entrepreneur (average of 10.75%) in the low technology stratum, and for the strata for medium and high technology, the third item was labor (averages of 8.46% and 8.09% for medium and high technological levels, respectively). Such results indicate the success of the “Balde Cheio” project on the strata analyzed, as all DUs had dairy cows with high production efficiency, focus on diluting fixed costs through herd structuring, sustainable technology, productive occupation of land, and raising the production scale, whose technological developments took place harmoniously with the time of assistance that the DUs have by extension technicians of the project.

Key Words: administration, efficiency indicator, production cost

2431 Integrating wearable sensors, automated milking systems, and weather station data for milk yield prediction in dairy cows. B. B. de Medeiros^{*1}, J. M. Hooker¹, C. Saha¹, T. Abdulrahman¹, and A. A. C. Alves^{1,2}, ¹*Department of Animal and Dairy Science, University of Georgia, Athens, GA*, ²*Institute for Integrated Precision Agriculture, Athens, GA*.

This study investigated the association between data collected from wearable sensors, automated milking systems (AMS), and weather stations, evaluating their impact on the prediction accuracy of the next 7-d average milk yield using different models trained on short-term time-series data. Data were collected from 1,312 lactating Holstein cows in a commercial dairy farm in Chowchilla, CA, using a freestall system with controlled temperature, robotic milking, and balanced partial mixed ration (PMR). The dataset included 326,204 observations of daily milk yield (DMY), electrical conductivity (EC), milk flow rate (FR), and number of milkings (NM), along with 363,554 observations of rumination and activity times from collar-mounted sensors. Climate variables were obtained from a nearby weather station to calculate the temperature-humidity index (THI). We extracted time-series features from 14 d interval from different data to predict the next 7-d DMY average (DMY7). The baseline model included the effects of DIM, lactation number, month, year, and reproduction status. Models compared were linear regression (LR), gradient boosting machine (GBM), and random forest (RF). We used a paired *t*-test to infer differences between prediction ability metrics according to the used dataset. With no previous DMY information, we observed that combining collar and climate data significantly increased the average R^2 ($P < 0.0001$) and reduced the mean absolute error ($P = 0.0001$) of DMY7 predictions across models. The average R^2 ranged from 0.32 (baseline) to 0.41 (collar and climate data combined). Including previous information for DMY was responsible for the most prominent R^2 increase in DMY7 prediction (0.32 to 0.83), with no substantial R^2 differences after DMY inclusion. However, we

observed a significant reduction in prediction error ($P = 0.0006$) when incorporating climate, collar, and AMS data along with DMY. Our results indicate that collar and climate information are especially important predictors of milk yield in the absence of historical milk yield data.

Key Words: rumination, machine learning, milk yield prediction

2432 Factors associated with milking speed at different stages of lactation in Holstein cows. M. B. Ugarte Marin^{*1}, V. A. B. Primo¹, R. C. Chebel^{1,2}, and R. S. Bisinotto¹, ¹*Department of Large Animal Clinical Sciences, D. H. Barron Reproductive and Perinatal Biology Research Program, University of Florida, Gainesville, FL*, ²*Department of Animal Sciences, University of Florida, Gainesville, FL*.

Objectives were to evaluate the association between milking speed and milk yield (MY) across different days in milk (DIM) intervals. Data were collected from a single dairy from September to December 2024 (ID; lactation number; DIM; date, time, and duration of milking; MY). Milking speed (kg/min) was calculated as MY divided by milking duration. Holstein cows were milked thrice daily in a 120-stall rotary parlor. A linear mixed effects model with repeated measures was built on R to evaluate average daily milking speed at 60-d intervals (10–70 DIM, $n = 379$; 71–130 DIM, $n = 1,337$; 131–190 DIM, $n = 869$; 191–250 DIM, $n = 590$; 251–310 DIM, $n = 204$) using the lme4 package and including cow as random effect. Models included the fixed effects of lactation group (2, 3, 4, 5+), DIM (linear and quadratic), daily MY, and the interactions between DIM (linear and quadratic) and MY. Milking speed was affected ($P < 0.001$) by the interaction between DIM² and MY in all intervals, except for 191–250 DIM when the milking speed was affected ($P < 0.001$) by the interaction between DIM and MY. These interactions indicate that, within each DIM interval (i.e., 10–70, 191–250, and 251–310 DIM), cows with greater daily MY maintain milking speed longer and exhibit a less pronounced decrease in milking speed as DIM progressed compared with cows with smaller MY. For 71–130 and 131–190 DIM, milking speed in cows with greater MY increased as DIM progressed, whereas it decreased in cows with smaller MY. Milking speed 10–70 DIM was associated ($P = 0.01$) with lactation group, as it increased 0.2 ± 0.1 , 0.3 ± 0.1 , and 0.2 ± 0.1 kg/min for 3, 4, and 5+ lactation cows compared with cows in lactation 2. Lactation group was not associated ($P \geq 0.06$) with milking speed at 71–130, 131–190, and 251–310 DIM. At 191–250 DIM, on the other hand, milking speed decreases 0.2 ± 0.1 , 0.3 ± 0.1 , and 0.2 ± 0.1 kg/min for 3, 4, and 5+ lactation cows compared with cows in lactation 2. Spearman coefficients depicted moderate correlation for milking speeds measured within cow at 30-d ($\rho = 0.49$ – 0.70) and 60-d intervals ($\rho = 0.45$ – 0.60), supporting management strategies focused on individual milking speed.

Key Words: milkability, milk flow, milking parlor

2433 Improving dairy cattle productivity in Nigeria: Physiological and morphological variations in White Fulani, Jersey, and their F₁ crossbred calves. H. Okin^{*1,2}, O. Jimoh³, and A. Adeyoye⁴, ¹*Department of Dairy Science, University of Abuja, Abuja, Nigeria*, ²*Veterinary and Livestock Services, State Ministry of Agriculture, Kaduna, Nigeria*, ³*Department of Agricultural Technology, The Federal Polytechnic Ado-Ekiti, Ekiti, Nigeria*, ⁴*Department of Animal Production, University of Ilorin, Ilorin, Nigeria*.

Dairy production in Nigeria faces challenges such as low milk yield, poor adaptability, and disease susceptibility in local and exotic cattle breeds. The White Fulani, a dominant indigenous breed, exhibits resil-

ience to heat and diseases but has lower milk productivity, whereas the high-yielding Jersey breed struggles with environmental stressors. Cross breeding the local and exotic cattle aims to combine the adaptability of White Fulani with the productivity of Jersey to improve dairy cattle performance. Understanding physiological and morphological variations among these breeds and their crossbred progeny is essential for optimizing breeding strategies, enhancing milk production, and ensuring sustainable dairy farming in Nigeria. This study evaluated physiological and morphological variations among White Fulani, Jersey, and their F₁ crossbred calves at Shonga Dairy Farm, Nigeria. Growth traits were recorded from 21 calves, and blood samples were collected over 6 mo to assess biochemical and hematological parameters. Results indicated no significant differences ($P > 0.05$) in most biochemical parameters among breeds, except for lower urea levels in Jersey compared with F₁ crossbreds. The F₁ crossbreds displayed intermediate linear body measurements but had a significantly higher heart girth ($P < 0.05$) than Jersey calves. Strong positive correlations were observed between heart girth and traits such as wither height, hip width, top line, and head length, suggesting their usefulness in estimating live weight. The alanine aminotransferase/aspartate aminotransferase ratios varied significantly ($P < 0.05$) across months. In contrast, hematological parameters were broadly similar, except for lower erythrocytic indices (MCV, MCH, and MCHC) in White Fulani and Jersey compared with F₁ crossbreds in some months. These variations were influenced by genetics and could be modulated by environment, management, and nutrition. Findings from this study provide valuable insights for trait selection in crossbreeding programs, supporting efforts to enhance dairy cattle productivity and adaptability in Nigeria.

Key Words: dairy, crossbreeding, productivity

2434 Production cost and number of lactations for the repayment period of dairy heifers. L. H. R. Silva¹, K. R. Oliveira^{*1}, A. L. Silva¹, J. V. C. Rodrigues¹, W. P. F. Amorim¹, M. I. Marcondes², T. E. da Silva³, J. H. C. Costa³, and P. P. Rotta¹, ¹Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil, ²William H. Miner Agricultural Research Institute, Chazy, NY, ³University of Vermont, Burlington, VT.

The objective of this study was to estimate the average number of lactations required to achieve the rearing repayment period. This study used a database containing financial and zootechnical information from 2021. The data were generously provided by the EDUCAMPO/SEBRAE group and included 311 farms in Brazil. Dairy farms were categorized into 3 milk production tiers based on average milk yield: low production tier (LOW; $n = 78$) with an average of 12.0 L/cow per day (range: 7.4–14.5 L); intermediate production tier (INT; $n = 155$) with an average of 18.0 L/cow per day (range: 14.6–22.5 L); and high production tier (UPP; $n = 78$) with an average of 26.7 L/cow per day (range: 22.6–32.0 L). A Monte Carlo simulation was employed to estimate the repayment period for each production tier, considering the following parameters: (1) rearing cost from birth to calving: US\$2,006.4 $\pm$ 62.52 (range: 874.6–6,048.6) for LOW, US\$1,821.4 $\pm$ 44.35 (range: 962.9–3,163.7) for INT, and US\$ 1,884.6 $\pm$ 62.52 (range: 1,205.4–2,872.9) for UPP; (2) milk net margin of 25%, 30%, and 35% $\pm$ 0.03% for LOW, INT, and UPP, respectively; (3) milk price received: US\$0.50 $\pm$ 0.001 for LOW and INT, and US\$0.51 $\pm$ 0.001 for UPP; (4) culling rate of 15%, 20%, and 25% for LOW, INT, and UPP, respectively; (5) price per 100 kg (arroba, live cattle weight unit for 30 kg) of culled cows: US\$30.49 across all tiers; (6) market price of male calves at birth: US\$20.32 for LOW and INT, and US\$10.16 for UPP; and (7) market price of female calves at birth: US\$30.49, \$40.65, and \$60.98 for LOW, INT, and UPP, respectively. The Monte Carlo simulation was conducted with 5,000

iterations using Latin Hypercube Sampling in ModelRisk. On average, the number of lactations required to achieve the repayment period was 3.98 (range: 1.33–9.33) for LOW, 2.64 (range: 1.33–6.33) for INT, and 1.64 (range: 0.66–3.66) for UPP. In conclusion, rather than solely evaluating the rearing cost of a heifer from birth to calving, it is essential to determine the number of lactations required to reach the breakeven point. This analysis should be conducted based on the specific production system and productivity level of each dairy farm.

Key Words: heifer, cost of production, repayment period

2435 Individual and combined associations of metritis and clinical endometritis with dairy cow profitability. O. A. Ojeda-Rojas^{*1}, J. Pérez-Báez², S. Casaro¹, F. Cunha¹, R. C. Chebel¹, A. De Vries³, J. E. P. Santos^{3,4}, F. S. Lima⁵, P. Pinedo⁶, G. M. Schuenemann⁷, R. C. Bicalho⁸, R. Gilbert⁹, W. W. Thatcher^{3,4}, and K. N. Galvão^{1,4}, ¹Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL, ²Escuela Medicina Veterinaria, Universidad Autónoma de Santo Domingo, Santo Domingo, Republica Dominicana, ³Department of Animal Sciences, University of Florida, Gainesville, FL, ⁴D. H. Barron Reproductive and Perinatal Biology Research Program, University of Florida, Gainesville, FL, ⁵Department of Population Health and Reproduction, University of California, Davis, CA, ⁶Department of Animal Sciences, Colorado State University, Fort Collins, CO, ⁷Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, ⁸FERA Diagnostics and Biologicals Corp., College Station, TX, ⁹School of Veterinary Medicine, Ross University, Basseterre, St. Kitts, West Indies, ¹⁰Department of Animal Sciences, University of Illinois, Urbana-Champaign, IL, ¹¹College of Veterinary Medicine, Texas A&M University, College Station, TX, ¹²Department of Animal Sciences, University of Wisconsin, Madison, WI.

The objective was to evaluate the individual and combined associations of metritis (MET) and clinical endometritis (CE) with dairy cow profitability. Cows ($n = 11,051$) from 16 dairy herds located in 4 regions of the US were classified as having no uterine disease (NUD), only MET, only CE, or both MET and CE (METCE). Incomes were calculated from milk yield, cow sales, and residual cow value at 305 DIM. Expenses were calculated from feed, reproductive, and replacement costs. Gross profit was calculated as the difference between incomes and expenses. Continuous and dichotomous outcomes were analyzed using mixed effects models. The additive association of MET and CE was evaluated using a nonorthogonal contrast. Cows with METCE produced less ($P < 0.05$) milk than cows with MET, CE, and NUD (9,514 vs. 9,969 vs. 9,919 vs. 10,109 kg/cow, respectively), which led to lesser ($P < 0.05$) milk sales for METCE than MET, CE and NUD (4,629 vs. 4,850 vs. 4,825 vs. 4,918 \$/cow, respectively). The proportion of pregnant cows at 305 DIM was lesser ($P < 0.05$) in cows with METCE (67%) than in cows with MET (76%) and CE (75%), which were lesser ($P < 0.05$) than in cows with NUD (82%). No difference ($P > 0.05$) was observed between cows with MET and CE. The proportion of cows leaving the herd by 305 DIM was greater ($P < 0.05$) in cows with METCE (38%) than in cows with MET (30%) and CE (31%), which were greater ($P < 0.05$) than in cows with NUD (25%). No difference ($P > 0.05$) was observed between cows with MET and CE. Overall, cows with METCE had lesser ($P < 0.05$) gross profit per cow (\$3,628) than cows with MET (\$3,905) and CE (\$3,921), which were lesser ($P < 0.05$) than NUD cows (\$4,085). No difference ($P > 0.05$) was observed between cows with MET and CE. Uterine diseases negatively affected the profitability of dairy cows by being associated with decreased milk yield, reproductive efficiency, and herd survival. The combination of MET and CE led to

negative additive associations with the overall performance and profitability of dairy cows.

Key Words: economics, uterine disease, dairy cow

2436 Development of milking characteristics in German Fleckvieh from 2000 to 2023. A. Schmutz¹, F. Grandl¹, and R. M. Bruckmaier^{*2}, ¹Bavarian Dairy Herd Improvement Association, Munich, Bavaria, Germany, ²Vetsuisse Faculty, University of Bern, Bern, Switzerland.

In Bavaria, a state in the south of Germany, the regular dairy herd improvement recording and milk sampling has been consistently performed with a mobile milk flow recording and sampling device (Lactocorder, WMB AG, Balgach, Switzerland) since 1998. As in most dairy cows worldwide, efficient animal breeding caused a considerable increase in milk production also in German Fleckvieh (FV) in Bavaria (305-d production: 6,077 kg in 2000 vs. 7,747 kg in 2023). The large number of Lactocorder milk flow recordings enabled us to test the hypothesis that milking characteristics have changed along with the increased production. We analyzed milk flow recordings from 3.2 Mio cows obtained from 2000 to 2023 in twice daily milked FV cows. To estimate changes in various milking characteristics, we ran multiple regression models and estimated marginal means of these characteristics in 2000 and 2023. The observed increase in milk yield (MY) of individual milkings from 11.47 to 11.87 kg (+3.5%) did not result in a proportionally longer main milking time (MMT, time from start of milk flow until decline to 200 g/min; 5.92 to 6.08 min [+2.7%]) because of a substantial increase in peak flow rate (PFR) from 2.80 to 3.19 kg/min (+13.9%) and to some extent also average milk flow (AMF) from 1.83 to 1.97 kg/min (+7.7%) from 2000 to 2023. The uneven distribution of milk among the udder quarters limits the plateau phase of milk flow (PP), which is determined by the quarter with the shortest duration of milk flow. Milk flow cessation in the first quarter determines the end of PP and the start of the decline phase (DP) and milking on empty teats. Most likely, the increased milk production and larger amount of milk stored in the udder in 2023 compared with 2000 and hence higher intra-mammary milk pressure and anatomical changes of the milk drainage system in the udder have caused a higher PFR during PP. Depending on the quarter distribution of milk storage in the udder the portion of PP and DP may have varied and affected AMF to a different extent. The

reason for a decreased ratio of PP and an increase of DP (−7% PP vs. +2.3% DP relative to MMT) remains unclear.

Key Words: dairy cow, milk flow, milking characteristics

2437 Economic and environmental performance of organic grass-fed dairies in the Northeastern US. K. V. Almeida^{*1}, H. M. Darby², S. E. Ziegler², S. Flack³, F. Ferreira⁴, and A. F. Brito¹, ¹Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, NH, ²University of Vermont Extension, Burlington, VT, ³Sarah Flack Consulting, Enosburg Falls, VT, ⁴Clean Air Task Force, Boston, MA.

Organic grass-fed (OGF) is the fast-growing segment of the organic dairy industry in the US. Therefore, it is important to understand their economic viability and environmental impact in the country. Data from 106 OGF dairies obtained from 6 Northeastern states (MA, NH, NY, NJ, PA, and VT) via a survey conducted in 2019 was used for calibrations of the Integrated Farm System Model 4.6. Production and economic data from 21 OGF farms accessed through the Dairy TRANS 4.4 tool were used in a hierarchical cluster analysis. Two clusters were generated based on farm area, herd size, production (e.g., milk production), and economic metrics (e.g., livestock sales, machinery expense, hired labor expense, property taxes, total expenses, total income, net cash income) as shown in Table 1. All analyses were performed in R Studio, and clusters were compared via ANOVA. Despite higher total expenses, cluster 2 farms had greater milk production, total income, and net cash income than cluster 1. Furthermore, the carbon footprint of cluster 2 farms was lower than that of cluster 1 because of increased milk production (kg/cow/year and kg/ha). In summary, cluster 2 OGF dairies were more profitable and emitted less carbon compared with cluster 1, with these responses mostly driven by improved productivity.

Key Words: carbon footprint, dairy cow, profitability

2438 Developing an understanding of Ontario dairy veterinarians when recommending care and management for down dairy cattle: A qualitative study. J. E. Brindle^{*}, C. B. Winder, D. B. Haley, T. F. Duffield, and D. L. Renaud, *University of Guelph, Guelph, ON, Canada.*

Table 1 (Abstr. 2437). Economic metrics and environmental performance of organic grass-fed dairies

Economic analysis ¹	Mean ± SEM		P-value
	Cluster 1 (n = 16)	Cluster 2 (n = 5)	
Area, ha	152 (20)	41 (5)	<0.01
Lactating cows, n/herd	56 (5)	50 (8)	0.52
Milk production, kg/ha	1,577 (239)	5,870 (196)	<0.01
Milk production, kg/cow/year	3,549 (207)	4,890 (282)	<0.01
Milk price, \$/kg	0.83 (0.02)	0.84 (0.01)	0.99
Total income, \$/ha	1,528 (226)	5,184 (252)	<0.01
Total expenses, \$/ha	1,004 (133)	3,083 (413)	<0.01
Net cash income, \$/ha	520 (124)	2,094 (369)	<0.01
Greenhouse gas emissions, kg of CO ₂ eq/year	Mean		± SD
Animal emissions	290,653	277,128	2,162
Carbon sequestration	−95,103	−97,195	4,751
Carbon footprint w/o biogenic CO ₂ eq, kg/kg FPCM	1.47	1.40	0.03
Carbon footprint w/ biogenic CO ₂ eq, kg/kg FPCM	1.14	1.08	0.04

¹CO₂eq = carbon dioxide equivalent; FPCM = fat and protein corrected milk (4% fat and 3.3% protein).

The objective of study was to examine Ontario dairy veterinarians' perspectives when working with producers on managing and caring for down dairy cows, focusing on barriers, protocols, current practices, and implementing best practices. A total of 20 one-on-one online interviews with dairy veterinarians were conducted between July to December 2024, lasting a median of 30 min (range: 20–45). In the interviews, veterinarians were asked about veterinarian-client relationships, thoughts on best management practices, protocol development, challenges for producers when adopting new practices, and about facilities and equipment for down cow care. The interviews were recorded, transcribed, and evaluated using applied thematic analysis in Quirkos (version 2.5.3, Quirkos Ltd.). Four themes emerged from the data: producer traits and decision making; veterinarian-client relationships; care and management practices; and facilities and equipment. Veterinarians described the influence of producer attitudes on decision making, with proactive producers implementing preventive strategies, whereas others exhibited resistance to change, often shaped by multigenerational traditions and economic constraints. Trust and communication emerged as critical components of veterinarian-client relationships, although it was acknowledged that these relationships take time to build. In terms of care and management, veterinarians emphasized timely intervention and welfare-focused approaches (e.g., providing pain medications and ensuring cow comfort). Facilities and equipment were identified as major constraints, particularly in older barns with limited space and accessibility, affecting the ability to provide optimal down cow care. Veterinarians also discussed euthanasia from the emotional challenges for producers to the practical criteria used to decide when to euthanize a down cow. By highlighting the complexities of producer decision making, veterinarian-client relationships, and care practices, this study provides insights into the factors influencing down cow management. These findings offer practical guidance for understanding the role of the veterinarian in the complex factors influencing dairy producers' management and care for down dairy cows.

Key Words: management practices, recumbent, qualitative

2439 A system dynamics approach to enhancing sustainability in dairy systems: Engineering the water-food-energy nexus for US dairy farms. A. Pushkaran*, S. Paudyal, and K. Kaniyamattam, *Department of Animal Science, Texas A&M University, College Station, TX.*

Milk production in the United States is often constrained by resource availability and exacerbated by environmental pressures. Our objective was to develop a system dynamics modeling approach to analyze the interrelationships between water use, energy consumption, and food production in a representative mid-size Texan dairy farm. The model incorporates critical feedback loops, including feed efficiency, manure management, water recycling, and renewable energy adoption, to identify leverage points for enhancing energy and water-related sustainability metrics. Key parameters such as feed availability, water demand, energy requirements, greenhouse gas emissions, and economic drivers for an average herd size of 1,000 milking cows were integrated into causal loop diagrams to map reinforcing and balancing feedback

mechanisms. Data sources included USDA reports, farm records, and publicly available environmental data customized for dairy farming. The model was validated using historical farm data from Texas-based dairy farms and stakeholder feedback. By simulating various scenarios (Table 1), we evaluated pathways to enhance resource efficiency, reduce environmental impact, and improve economic resilience. The findings (Table 1) highlight the necessity of a systems-based approach, emphasizing the need for integrated strategies that balance productivity with resource conservation. The research underscores the role of renewable energy integration, precision irrigation, and nutrient recycling in reducing dependency on finite resources. By offering a decision support tool for policymakers, industry stakeholders, and researchers, this study provides actionable insights for designing resilient, resource-efficient dairy systems that can adapt to changing environmental and economic conditions.

Key Words: dairy sustainability, water-food-energy nexus, system dynamics

2440 An economic simulation model to assess the effect of the 21-d pregnancy rate, semen type, and heifer retention rate on the optimal insemination eligibility period for lactating dairy cows. M. R. Lauber*, V. E. Cabrera, and P. M. Fricke, *University of Wisconsin–Madison, Madison, WI.*

We developed a discrete, monthly Markov chain model to simulate a 1,000-cow herd to assess the herd dynamics and economic performance of scenarios with varying 21-d pregnancy rates (21-d PR), semen types, and insemination eligibility periods (IEP). We categorized the estimated herd 21-d PR as low (20%), below average (25%), average (30%), above average (35%), or high (40%). Semen types were (1) conventional semen for all inseminations and parities (CON) and (2) sexed semen inseminations for heifers (75%) and for first and second parity cows (first and second artificial insemination) with the remainder inseminated with beef semen (S+B). We varied the IEP for cows from 50 to 260 d to determine an optimal IEP defined as the period with the maximum net return (NR) with sufficient replacements to maintain herd size. Treatments included varied beef calf market values (\$385 to \$1,125), heifer retention rates (HRR; 75% to 90%), and 21-d PR (20% to 40%). The optimal IEP in CON scenarios varied from 80 to 170 d for high to low herds but was 200 d for S+B herd scenarios regardless of the 21-d PR. As expected, increasing the beef calf market value increased NR. The optimal IEP increased as the HRR decreased for CON scenarios with a low herd with a 75% HRR producing insufficient replacements. In S+B scenarios, the optimal IEP remained 200 d regardless of HRR and 21-d PR, except for low and below-average herds with 75% to 80% or 75% HRR, respectively, because of insufficient replacements. The base NR (20% 21-d PR) was \$50.07 greater per cow per year for S+B than CON scenarios with a 170 d IEP. Further, the NR increased from \$5.63 to \$2.53 and \$7.39 to \$2.33 per 1 percentage point increase in the 21-d PR for CON and S+B scenarios with a 170 d IEP, respectively. We conclude that the optimal IEP depends on the herd 21-d PR, semen type, and HRR and that increasing the 21-d PR increases NR regardless of semen type. This work was supported by USDA NIFA CARE project

Table 1 (Abstr. 2439). Summarizing simulated outputs from the scenarios based on parameters for a 1,000-cow dairy operation in Texas

Scenario	Water usage	Energy usage	GHG emissions	Economic impact
Technological advancements in energy efficiency	No significant change	30% reduction	15% reduction	10% increase in net revenue
Improvements in water management	40% reduction	5% increase	5% reduction	3% increase in revenue
Climate adaptation strategies	25% reduction	10% reduction	20% reduction	7% increase in revenue

2021-68008-34105 and USDA NIFA AFRI Predoctoral Fellowship 2024-67011-42904.

Key Words: sexed and beef semen, 21-d pregnancy rate, dairy economics

2441 Effect of feeding microencapsulated botanicals and organic acids to lactating Holstein cows under heat stress conditions on milk production performance and feed efficiency: A pen-level randomized controlled trial. M. Thomas¹, J. Moncada Torres¹, V. Sáinz de la Maza-Escolà², A. Piva^{2,3}, E. Grilli^{3,4}, P. Lira¹, J. Venegas Flores¹, and M. Stangaferro^{*1,5}, ¹*Dairy Health and Management Services, Lowville, NY*, ²*Vetagro Spa, Reggio Emilia, Italy*, ³*University of Bologna, Bologna, Italy*, ⁴*Vetagro Inc., Chicago, IL*, ⁵*Universidad Nacional del Litoral, Esperanza, Argentina*.

The objective of this study was to evaluate the effect of feeding a microencapsulated blend of botanicals and organic acids (AviPlus R, Vetagro Spa, Reggio Emilia, Italy) on milk production performance and feed efficiency of Holstein cows naturally exposed to heat stress conditions. The trial was conducted in 2 commercial dairy farms in Torreon, Mexico, from June to September 2024. Cows (n = 2,672) were enrolled and assigned to the study pens (n = 10; 5 control and 5 treatment) balanced for parity, milk yield, and DIM. Pens were fed a

total mixed ration (TMR) diet either without (Control) or with AviPlus R supplementation (25 g/cow/d). The study included 1 wk of baseline period (covariate), 2 wk of adaptation, and 8 wk of data collection. Cow-level milk yield, body condition score (BCS), health outcomes and body weight (BW, n = 688, primiparous only) data, and pen-level energy-corrected milk (ECM), dry matter intake (DMI), feed efficiency (FE), and milk components were monitored. Statistical analyses included mixed linear and logistic models accounting for repeated measures (when applicable) with treatment, time (wk of study), parity, and their interactions as fixed effect. In all models, pen was the experimental unit (pen nested within farm and treatment included as a random effect), and the observational unit (pen vs. the individual animal) varied with the outcome assessed. Milk production, protein percent, DMI, ECM, BCS and health events were similar between groups. Pens fed AviPlus R had greater ($P \leq 0.05$) milk fat percent on wk 10–11 and total solids percent on wk 9–11 than control. Fat yield (1.21 vs. 1.14 ± 0.01 kg/d) and FE (1.37 vs. 1.33 ± 0.01 kgECM/kgDMI) were greater ($P \leq 0.05$) for pens fed AviPlus R compared with control. Cows fed AviPlus R had lower BW on wk 5–6, but greater BW on wk 9–11 ($P \leq 0.01$). These findings suggest that AviPlus R supplementation may enhance production efficiency by improving FE and milk fat yield while supporting BW and BCS maintenance under heat stress conditions.

Key Words: heat stress, feed efficiency, milk fat

Production, Management, and the Environment: The DairyUp Project—Attacking Major Challenges for Dairy in a Multidisciplinary Project; Posters

2442 Genetic diversity of bovine rotavirus in dairy calves with and without diarrhea in New South Wales, Australia. Y. Kida^{1,4}, Z. U. Abedien^{1,4}, I. J. Lean^{2,4}, and B. P. Brito^{*3,4}, ¹*Australian Institute for Microbiology & Infection, University of Technology Sydney, Ultimo, NSW, Australia*, ²*Scibus, Camden, NSW, Australia*, ³*New South Wales Department of Primary Industries and Regional Development (DPIRD), Elizabeth Macarthur Agricultural Institute, Menangle, NSW, Australia*, ⁴*Dairy UP Program, Camden, NSW, Australia*.

Bovine rotavirus (BRV) is a major cause of diarrhea in calves, resulting in significant economic losses in the livestock industry. To comprehensively assess the diversity of BRV circulating in New South Wales (NSW), we conducted a large-scale study to identify both dominant and less common genotypes and evaluate their association with disease status and age. A total of 593 rectal samples were collected from calves on 72 farms across NSW. Rectal swabs from 349 healthy calves, 154 with diarrhea, 42 with respiratory issues, 11 with both symptoms, 30 with nonspecific symptoms, and 7 with unknown status. Total RNA sequencing was performed with a mean sequencing depth of 19 gigabases using an Illumina platform. The transcriptome was assembled de novo using SPAdes genome assembler v3.13.0, and the contigs were taxonomically classified using NCBI BLASTn. The transcriptome was mapped to the assembled contigs using Kallisto v0.5.1.1. To assess statistical differences between the contigs mapped to transcriptomes of calves with and without diarrhea, we used DESeq2 v1.44.0. Genotypes were predicted using an online Rotavirus A Genotyping tool developed by the National Institute for Public Health and the Environment, Netherlands. The most common genotypes were G10&6-P[5&11]-I2-R2-C2-M2-A3&13-N2-T6-E2-H3, with the most abundant combinations being G6P[5] and G10P[11]. Less frequent genotypes, such as G24 and P[33], as well as Rotavirus B, were also identified in at least 2 farms. Notably, almost all farms had at least one BRV-positive calf. While G10 was detected in 44 farms, G6 was found in only 25 farms. G24 was found in 2 farms, P[33] was observed in one farm, and Rotavirus B was detected in 5 farms. Multiple co-infections involving different genotype combinations were also observed, with G10 and G6 in 9 farms, as well as P[11] and P[5] in 4 farms. The abundance of the nonstructural protein 3 gene showed a strong, significant association with diarrhea, as did viral protein 7 genotypes G10 and G6. Our findings highlight the high prevalence of 2 dominant genotypes of BRV in calves with clinical and subclinical infections.

Key Words: rotavirus, diarrhea, metagenome

2443 Greenhouse gas emissions of confinement and pasture-based dairy farms: Implications for mitigation. M. F. Dida^{*1,2}, S. C. Garcia^{1,2}, and L. A. Gonzalez^{1,2}, ¹*School of Life and Environmental Sciences, Faculty of Science, and Sydney Institute of Agriculture, The University of Sydney, Camden, NSW, Australia*, ²*Dairy UP Program, Camden, NSW, Australia*.

Despite extensive research on the environmental impacts of dairy farming, comparative greenhouse gas (GHG) emissions from confinement and pasture-based systems are still unclear due to inconsistent results from previous studies, which also did not account for carbon (C) sequestration by trees. The present study aimed to compare the GHG emissions of 2 Australian milk production systems (confinement and pasture-based, with 5 farms in each system) using a life cycle assessment approach that

incorporates C sequestration by trees. The confinement system used a total mixed ration, whereas the grass-based system primarily relied on grazed forage with concentrate supplementation. The Australian Dairy Carbon Calculator, a Tier 3 tool, was employed to predict the total GHG emissions and emission intensity for milk production. This calculator incorporates emission factors and methodologies from the National Greenhouse Gas Inventory and Intergovernmental Panel for Climate Change, as reported to the United Nations Framework Convention on Climate Change. The tool calculates the emission intensity of a milk production system in terms of CO₂ equivalents (CO_{2eq}), with emissions allocated to both milk and meat. Animal emissions dominated both systems, representing 85% (54% from enteric CH₄ and 31% from manure) of total emissions in the confinement system and 71% (58% from enteric CH₄ and 13% from manure) in the pasture-based system. Notably, the confinement system had 13% lower enteric CH₄ intensity and 88% lower prefarm embedded intensity (kg CO_{2eq}/kg FPCM) compared with the pasture-based system. However, manure-related GHG emission intensity was 129% higher in the confinement. The confinement and pasture-based systems had similar emission intensity for milk (1.02 ± 0.038 vs. 1.07 ± 0.069 kg CO_{2eq}/kg FPCM) and meat (5.51 ± 0.779 vs. 6.76 ± 0.868 kg CO_{2eq}/kg liveweight). Targeted mitigation strategies should be implemented for each production system to reduce emissions from manure in confinement systems but pre-embedded and fertilizer emissions in pasture-based systems, but enteric CH₄ is predominant in both systems.

Key Words: dairy, methane, nitrous oxide

2444 Dairy farming system and land use influence soil organic carbon stocks. M. F. Dida^{*1,3}, B. Minasny², S. C. Garcia^{1,3}, and L. A. Gonzalez^{1,3}, ¹*School of Life and Environmental Sciences, Faculty of Science, and Sydney Institute of Agriculture, The University of Sydney, Camden, NSW, Australia*, ²*Sydney Institute of Agriculture, School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, NSW, Australia*, ³*Dairy UP Program, Camden, NSW, Australia*.

The intensification of agricultural practices has profound ecological implications, including adverse effects on carbon (C) sequestration and biodiversity. In dairy farming, intensification drives changes in land use toward more cropping, which can alter soil properties and soil organic C (SOC). Given these impacts, understanding the interplay between dairy farm farming system, land use, and soil characteristics is essential for developing sustainable on-farm land uses that enhance C storage and potentially offset greenhouse gas (GHG) emissions. Consequently, the present study aimed to examine SOC and other soil physicochemical properties across 6 common land use practices on confinement and pasture-based commercial dairy farms: improved pasture (IP), natural pasture (NP), mixed pasture-cropping (MPC), continuous forage cropping (CFC), tree areas (TA), and built-up areas (BA) with no grazing or cropping. A total of 810 soil samples were collected from 9 commercial dairy farms in New South Wales, Australia, at a depth of 0–30 cm. A mixed-effects model was used to assess the effects of land use type and farming system on soil physicochemical properties. SOC stock was highest in NP, followed by TA, IP, MPC, and CFC, with the lowest values in BA ($P < 0.001$). Land use type influenced total nitrogen stock ($P < 0.001$), but total phosphorus was not affected ($P > 0.05$). Pasture-based systems had higher ($P < 0.05$) SOC (105.0 ± 6.26 vs. 60.0 ± 7.00 Mg

C ha⁻¹) and total nitrogen stock (8.73 ± 0.654 vs. 5.30 ± 0.731 Mg N ha⁻¹), whereas total phosphorus stock (0.56 ± 0.118 vs. 0.15 ± 0.105 Mg P ha⁻¹) was greater ($P < 0.05$) in confinement systems. Overall, the NP, IP, and TA land use types, along with pasture-based dairy farming systems, showed greater SOC stocks. These results suggest that both farming systems and land use types could play a role in offsetting GHG emissions and contribute to climate change mitigation. However, further studies are needed to assess annual C sequestration rates for each farming system and land use type to quantify their potential for offsetting GHG emissions within dairy farms.

Key Words: dairy, confinement, pasture

2445 Factors influencing lactose production in dairy cows. J. I. Gargiulo^{*1,4}, S. C. Garcia^{2,4}, and R. C. Hovey^{3,4}, ¹New South Wales Department of Primary Industries and Regional Development, Menangle, NSW, Australia, ²Dairy Science Group, School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Camden, NSW, Australia, ³Department of Animal Science, University of California–Davis, Davis, CA, ⁴Dairy UP Program, Camden, NSW, Australia.

Lactose is the primary carbohydrate in bovine milk and determines milk volume by influencing its water content. The concentration of lactose, along with other milk solids, varies across species and cow breeds. However, large-scale data on lactose are scarce, and no studies have examined the factors influencing its variability and production in Australia. We analyzed 393,772 herd test records from 33,280 cows across 85 farms in New South Wales (2008–2022), to assess the relationship between lactose output and milk yield (MY), composition and quality, genetics, and environmental factors. We used Pearson correlations and linear mixed modeling to examine the effects of parity, lactation stage, breed, year, season, climate, heritability, as well as differences between the overall population and cows consistently producing higher milk solids yield (MSY, fat yield + protein yield) per unit of lactose yield (LY). LY, lactose percentage (LP), and MSY varied significantly by breed, parity, lactation stage and season. Both LY and LP peaked in early lactation and declined as lactation progressed, consistent with the traditional MY curve. LY increased with parity number while LP decreased. Lactose yield was negatively correlated with milk fat and protein percentages and environmental factors such as the temperature-humidity index, whereas LP was negatively associated with SCC. Holsteins had the highest daily LY (1.24 kg) and MSY (1.80 kg) but the lowest LP (4.95%), while Jerseys produced the lowest LY (0.93 kg) and MSY (1.57 kg) and had the highest MSY:LY. Heritability estimates suggested a moderate genetic influence on LY, LP, and MSY:LY (0.24–0.33). Cows with higher MSY:LY produced 18% less LY and 15% less MY but maintained similar MSY. These cows were primarily Jerseys, Holsteins, and crosses, and were overrepresented as progeny of 13 sires. These findings highlight key factors influencing lactose production and identify cows that produce lower milk volumes while maintaining MSY, a trait aligned with local milk payment systems. Identifying these cows and their genetic backgrounds offers opportunities for targeted breeding programs to enhance milk production efficiency and composition while reducing environmental impact.

Key Words: lactose, variability, productivity

2446 Microbial diversity using meta-transcriptomics in dairy calves: An insight of the resistome and virulome in health and enteric disease. Z. U. Abedien^{1,4}, S. P. Djordjevic¹, I. J. Lean^{2,4}, J. Webster³, and B. P. Brito^{*3,4}, ¹Australian Institute for Microbiology

and Infection, University of Technology Sydney, Ultimo, NSW, Australia, ²Scibus, Camden, NSW, Australia, ³New South Wales Department of Primary Industries and Regional Development (DPIRD), Elizabeth Macarthur Agricultural Institute, Menangle, NSW, Australia, ⁴Dairy UP Program, Camden, NSW, Australia.

Microbial communities in dairy calves significantly impact their health, influencing the occurrence of enteric and respiratory diseases. A deeper understanding of microbial diversity in health and disease is critical for improving diagnostic and treatment approaches. This study aimed to explore the microbial diversity and gene expression patterns of various virulence and antimicrobial factors in rectal samples from dairy calves with varying health conditions and 4 different age groups sampled across 72 farms in New South Wales, Australia. A total of 593 rectal samples including 168 from calves with diarrhea were analyzed using high-throughput RNA sequencing. Bacterial taxonomy was assessed using MetaPhlAn 4, calculating α (Shannon and Simpson indices) and β (Bray–Curtis dissimilarity) diversity. Virulence factors and antimicrobial resistance gene expression were also assessed within each age group and disease status. The analysis revealed a complex microbial landscape characterized by approximately 1,000 distinct bacterial genera with top genera including *Anaerovibrio*, *Prevotella*, and *Segatella*. Healthy calves had significantly higher diversity compared with symptomatic calves. Differential expression analyses showed that although antimicrobial resistance genes (e.g., *cfxA*, *Sul2*, *TetQ*, *dfrA-12*, and *aph(6)-la*) were present across different age groups, they were significantly greater in calves with diarrhea compared with the healthy calves. Similarly, virulence factors such as *afaE VIII*, *espB*, *espD*, *ompA 28*, and *PorA* were distinctly elevated in symptomatic calves compared with asymptomatic calves. This study highlights the diversity of bacterial communities in dairy calves and their association with disease status in relation to virulence factors and antimicrobial resistance genes providing important insights that may inform future strategies for improving calf health and antimicrobial stewardship in the dairy industry.

Key Words: metatranscriptomics, virulence factor, enteric disease

2447 Potential of HSP70 as a noninvasive biomarker for detecting heat stress in dairy cow. M. R. H. Rakib^{*1,5}, V. Messina^{1,5}, J. I. Gargiulo^{2,5}, J. Nguyen^{1,5}, N. A. Lyons³, I. N. Pathirana⁴, and S. C. Garcia^{1,5}, ¹Dairy Science Group, School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Camden, NSW, Australia, ²NSW Department of Primary Industries and Regional Development, Menangle, NSW, Australia, ³DairyNZ, Hamilton, New Zealand, ⁴Department of Animal Science, Faculty of Agriculture, University of Ruhuna, Kamburupitiya, Sri Lanka, ⁵Dairy UP Program, Camden, NSW, Australia.

Heat stress (HS) negatively impacts dairy cows, leading to reduced milk production, impaired reproduction, and compromised welfare. Recently, the presence of heat shock protein 70 (HSP70) in bovine milk has been identified as a potential noninvasive biomarker for detecting HS in cows. This study aims to evaluate various methods for detecting HS in dairy cows, with a particular focus on assessing the effectiveness of HSP70 as an indicator. Data from 25 Holstein Friesian cows (third parity, 114 ± 8.3 DIM) in a pasture-based system were collected every 2 d over a 7-wk period at The University of Sydney's Dairy Farm, NSW, Australia. Variables included rectal temperature (RT), infrared thermography (IRT), milk HSP70 concentration (by competitive ELISA, sensitivity: 7.82–4,000 ng/mL), milk yield (L/day) and composition (protein, fat, SNF, lactose). Per-minute reticulorumen temperature (RRT) via bolus sensor and meteorological data from the nearest weather station were also collected over the period of the study. Pearson correlation analyses

and linear mixed models were used to assess the direct and lag effects of HS detection methods compared with the temperature humidity index (THI), the industry standard for assessing HS. Results revealed that milk HSP70 (ng/mL) was significantly ($P < 0.001$) correlated with THI, peaking at a 35-h lag, indicating a delayed response. Reticulorumen temperature correlated ($P < 0.001$) positively with THI at a 7-h lag, while RT and IRT showed immediate and consistent correlations ($P < 0.001$) from h 0. Milk yield exhibited a strong negative correlation ($P < 0.001$) with THI, declining from a 25-h lag and peaking at 48 h. However, protein (%), lactose (%), and SNF (%) showed significant (P

< 0.001) reductions at an 11-h lag, indicating a more immediate response compared with milk yield. Infrared thermography, RT, and RRT enable real-time and near-real-time HS monitoring, while milk HSP70 provides unique cellular-level insights. Integrating HSP70 with conventional methods can improve HS prediction accuracy, aid heat-tolerant cow selection, and enhance dairy cow welfare under thermal stress. Future research should assess individual variability in HSP70 expression among cows to optimize its use as a biomarker.

Key Words: animal welfare, detection method, heat shock protein

Ruminant Nutrition 2: General—Posters

2448 Effects of a magnesium phosphate source on lactating dairy cows. J. P. A. Rezende^{*1}, L. C. B. Pimenta¹, M. C. S. Santos¹, G. M. de Souza¹, C. Gousset², B. Ribeiro², and M. A. C. Danes¹, ¹Universidade Federal de Lavras, Lavras, MG, Brazil, ²Phosphea, Dinard, France.

Magnesium phosphate (MgP; Phosphea, France; 26% Mg, 14% P) is a potential Mg and P source, but requires evaluation. We investigated the effects of MgP on rumen pH, performance, milk fatty acids (FA) profile, total-tract digestibility (via total fecal collection), and fecal pH of lactating cows. Twenty-one individually housed Holstein cows (144 ± 77 DIM), in 3 × 3 Latin squares (21-d periods), received the 3 treatments: dicalcium phosphate (DCP) + magnesium oxide (MgO) supplying 0.54% P and 0.40% Mg on DM basis (CTL); MgP supplying the same levels of P and Mg as CTL (Iso-P&Mg); or MgP + MgO matching CTL's P but providing 0.53% Mg (Iso-P). Diets had 17.3% forage NDF, 20.5% physically effective NDF, 24.0% starch, 18.3% nonstarch NFC, and 5.0% EE. The statistical model included fixed effects of treatment and period, and random effects of square and cow(square). Time below pH 5.8 (284 ± 58 min/d), milk yield (34.5 ± 1.6 kg/d), and DMI (22.3 ± 1.0 kg/d) did not differ ($P > 0.20$). Milk fat and solids concentration were higher ($P \leq 0.05$) for Iso-P (3.60% ± 0.10% and 12.39% ± 0.15%, respectively) than CTL (3.45% and 12.26%) and Iso-P&Mg (3.44% and 12.24%). Milk de novo FA did not differ (0.83% ± 0.02% of milk, $P = 0.31$), but preformed FA were higher ($P \leq 0.04$) for Iso-P (1.41% ± 0.04%) than CTL (1.33%) and Iso-P&Mg (1.33%). Compared with Iso-P&Mg, Iso-P had higher digestibility of NDF (49.4% vs. 43.4% ± 1.6%, $P < 0.01$), EE (92.3% vs. 90.4% ± 0.5%, $P = 0.02$), and tended to have higher CP digestibility (75.2% vs. 73.0% ± 0.8%, $P = 0.07$). Compared with CTL, Iso-P had higher starch digestibility (99.3% vs. 98.9% ± 0.1%, $P = 0.04$) and tended to have higher digestibility of NDF (49.4% vs. 46.2% ± 1.6%, $P = 0.10$), and EE (92.3% vs. 90.9%, $P = 0.10$). Cows fed Iso-P had greater Mg digestibility (27.0% vs. 21.0% ± 2.2%, $P = 0.05$) than Iso-P&Mg. Cows fed Iso-P tended to have greater P digestibility (36.0% vs. 28.0% ± 2.2%, $P = 0.06$) than CTL. Fecal pH was higher for Iso-P (7.53 vs. 7.44 ± 0.02, $P = 0.03$) than CTL. Based on rumen pH, performance and digestibility, MgP can safely replace MgO and DCP. Higher milk fat concentration in Iso-P was linked to increased preformed FA, consistent with greater EE digestibility.

Key Words: mineral, magnesium phosphate

2449 Effects of source of trace minerals on blood metabolites and mineral status in transition dairy cows. Z. Sarwar^{*1}, T. M. Adeoti¹, M. N. Marinho¹, J. Rasia¹, M. C. Perdomo¹, T. Engle², K. E. Griswold³, and J. E. P. Santos¹, ¹University of Florida, Gainesville, FL, ²Colorado State University, Fort Collins, CO, ³Selko USA, Indianapolis, IN.

Objectives were to evaluate the effects of replacing sulfate (STM) with hydroxychloride (HTM) sources of Zn, Cu, and Mn on blood metabolites and mineral content in serum and liver in dairy cows. One-hundred forty-one Holsteins were blocked by lactation (0 vs. >0) and randomly assigned to STM ($n = 70$) or HTM ($n = 71$). Trace minerals supplemented in the basal diets resulted in Zn, Cu, and Mn concentrations of 64, 15, and 58 mg/kg of diet DM prepartum, and 77, 19, and 65 mg/kg diet DM postpartum, respectively. Treatments started at 245 d of gestation and stopped at 105 DIM. Blood was analyzed for BHB, NEFA, haptoglobin, and mineral concentrations on d -8, -5, -2, 6, 13,

and 23 relative to calving. Hepatic tissue collected at 10 and 50 DIM was assayed for minerals. Data were analyzed by mixed-effects models with the fixed effects of treatment and covariates and the random effects of block and cow nested within treatment and block. Linear regressions between intake of minerals and mineral content in serum and liver were performed. Results are in Table 1 with LSM of all time points pre- and postpartum. No associations were observed between prepartum intake of minerals and serum or liver mineral content ($R^2 < 0.01$; $P > 0.95$). Cows fed STM had greater concentrations of haptoglobin postpartum compared with cows fed HTM. Cows fed STM had greater concentrations of Zn and Cu, but smaller of Mn in serum prepartum compared with cows fed HTM. Treatment did not affect the contents of Zn and Mn in liver at 10 or 50 DIM; however, at 10 DIM, liver Cu tended to be greater for STM than HTM, whereas at 50 DIM liver Cu was less ($P = 0.02$) for STM than HTM fed cows (287 vs. 318 ± 9 mg/kg DM). Source of trace minerals had small effects on serum or liver mineral content.

Key Words: hydroxychloride, mineral, sulfate

2450 Evaluating the vinegar test as a practical method for assessing the solubility of alkalizing magnesium sources for dairy cattle. J. L. Bermeo^{*1}, N. Silva del Río^{1,2}, and P. K. Pandey², ¹University of California Davis Veterinary Medicine Teaching and Research Center, Tulare, CA, ²Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, CA.

One factor influencing the availability of supplemental Mg is its solubility in the rumen. While laboratory-based solubility tests provide valuable insights, they are often costly and time-consuming. A simpler alternative is the vinegar test which mixes 3 g of an alkalizing Mg source in 40 mL of acetic acid (pH ~2.45) and evaluates how much the pH changes as an indicator of quality. However, its reliability in ranking Mg sources and predicting solubility under rumen-like conditions remains uncertain. Therefore, this study aimed to evaluate commercial Mg sources available in US feed mills using the vinegar test at 0.5 h and Mg solubility analyzed by inductively coupled plasma mass spectrometry after 1 h of in vitro incubation at pH 6 (Universal Testing Laboratory, Quincy, IL). Eleven Mg sources were assessed (Table 1).

Table 1 (Abstr. 2449).

Item	Treatment		SEM	P-value
	STM	HTM		
Prepartum				
NEFA, mM	0.25	0.25	0.02	0.62
Haptoglobin, µg/mL	7.97	5.74	1.79	0.88
Serum Zn, mg/L	1.31	1.21	0.03	0.006
Serum Cu, mg/L	1.29	1.18	0.03	0.001
Serum Mn, µg/L	1.63	1.75	0.03	0.004
Postpartum				
NEFA, mM	0.50	0.52	0.04	0.53
BHB, mM	0.60	0.54	0.04	0.12
Haptoglobin, µg/mL	40.0	25.7	7.1	0.05
Liver Zn d 10, mg/kg DM	105	108	2.8	0.34
Liver Cu d 10, mg/kg DM	310	296	6.6	0.09
Liver Mn d 10, mg/kg DM	10.2	9.78	0.36	0.29

Table 1 (Abstr. 2450).

Source	pH vinegar test	Relative Mg solubility (%)
CaMg(CO ₃) ₂ -B	3.41	12
CaMg(CO ₃) ₂ -E	3.47	23
CaMg(CO ₃) ₂ -F	3.60	11
CaMg(CO ₃) ₂ -A	3.86	7
CaMg(CO ₃) ₂ -D	4.19	3
CaMg(CO ₃) ₂ -C	4.81	21
MgCO ₃ -B	5.17	90
MgO	6.54	38
MgCO ₃ -A	6.62	98
PMB	7.13	65
CaMg(OH) ₄	11.06	99

The pH increase ranged from 3.41 to 11.06, while Mg solubility varied from 3% to 99%. A simple linear regression analysis showed a positive relationship between pH increase in the vinegar test and Mg solubility ($R^2 = 0.60$; $P < 0.01$), suggesting that for every unit increase in pH, solubility increased by approximately 12.85%. However, pH increase did not always correspond to higher solubility. For example, CaMg(CO₃)₂-D displayed a pH of 4.19 but had the lowest solubility (3%), whereas CaMg(CO₃)₂-E had a slightly lower pH (3.47) yet the highest solubility among the sources that shared the same chemical composition (23%; Table 1). This highlights potential inconsistencies in using pH alone as a direct predictor of solubility, suggesting that while the vinegar test is a simple and reproducible tool for comparing alkalizing Mg sources, it could primarily reflect acid-buffering capacity rather than reflecting true solubility under rumen-like conditions.

Key Words: magnesium solubility, vinegar test, ruminant

2451 Optimal dose of cashew nutshell extract on milk and milk component production in lactating cows. S. E. Schuling*, D. E. Schimek, and M. Engeseth, *NutriQuest, Mason City, IA.*

Thirty-nine primiparous and 78 multiparous Holstein cows averaging (mean $\pm$ SD) 100 ± 58 DIM were used in a randomized complete block design to determine the optimal dose of cashew nutshell extract (CNSE; DynOmik; Adisseo) on milk and milk component production. Cows were blocked by parity, DIM, and milk production. Cows within block were randomly assigned to dietary treatments: 1) control diet (CON), 2) CON + 2.5 g/d CNSE (CNSE2.5) and 3) CON + 5 g/d CNSE (CNSE5.0). Cows were housed in a freestall barn equipped with 8 voluntary milking systems (VMS; DeLaval). Control diet contained 53% forage and was formulated for 26.6 kg DMI, and CNSE was included in the pellet fed at the VMS for CNSE2.5 and CNSE5.0. The partial mixed ration was fed 1 $\times$ and number of visits per cow per day was 2.78 ± 0.30 . Milk was sampled every 2 wk for analysis of components. Data were analyzed using the GLIMMIX procedure of SAS. There was no effect of treatment on milk protein content ($3.30\% \pm 0.02\%$), MUN (15.38 ± 0.20 mg/dL), or BW (710.4 ± 2.75 kg). There was a trend for a quadratic effect of treatment on milk yield ($P = 0.08$; mean $\pm$ SE; $47.86, 49.88, 47.17 \pm 1.14$ kg/d for CON, CNSE2.5, and CNSE5.0, respectively). There was a linear effect of treatment on milk fat content ($P = 0.04$; $4.10\%, 4.24\%$, and $4.29\% \pm 0.07\%$ for CON, CNSE2.5, and CNSE5.0, respectively). There was no effect of treatment on milk fat yield (2.00 ± 0.04 kg), but there was a quadratic effect of treatment on milk fat+protein yield ($P = 0.03$; $7.76, 8.28, 7.85 \pm 0.17$ kg, for CON, CNSE2.5, and CNSE5.0, respectively). De novo fatty acid content increased with CNSE ($P =$

0.03 ; $1.20, 1.26, 1.27 \pm 0.02$ g/100 g for CON, CNSE2.5, and CNSE5.0, respectively). Energy-corrected milk (ECM) yield increased quadratically with feeding CNSE ($P = 0.04$; $52.89, 56.06$, and 53.27 ± 1.09 kg/d for CON, CNSE2.5, and CNSE5.0, respectively). At 5.0 g/d CNSE, resulted in lower pellet intake ($P = 0.01$; quadratic; $6.00, 6.45$, and 5.77 ± 0.17 kg/d for CON, CNSE2.5, and CNSE5.0, respectively). Cashew nutshell extract fed at 2.5 g/d increased ECM yield, milk fat content, and pellet intake.

Key Words: cashew nutshell extract, milk yield, milk component

2452 Synbiotic supplementation improves feed efficiency in multiparous Holstein cows during peak lactation. W. S. Da Rosa, R. M. Da Rosa, L. L. Do Santos, T. C. Da Silva, U. S. Londero, E. R. Komminou, V. R. Rabassa, F. A. B. Del Pino, and M. N. Corrêa*, *Federal University of Pelotas (UFPEL), Teaching, Research, and Extension Center in Livestock (NUPEEC HUB), Pelotas, Rio Grande do Sul, Brazil.*

The pursuit of greater efficiency and profitability in dairy farming has driven the adoption of innovative nutritional strategies, such as synbiotics, combinations of probiotics and prebiotics that promote gut health and improve feed conversion. This approach has gained attention as an alternative to antibiotic growth promoters, a practice already banned in several countries and facing increasing restrictions. Despite the well-documented benefits of probiotics and prebiotics individually, research on synbiotics in dairy cows, particularly their effects on feed efficiency, remains limited. This study evaluated the impact of synbiotic supplementation on feed intake and efficiency in Holstein cows at peak lactation. The experiment was conducted on a commercial farm in Rio Grande do Sul, Brazil, using 30 multiparous cows between 60 and 120 d in milk, divided into 3 groups: Control (CON), receiving a standard total mixed ration (TMR); Treatment 10 g (T10), receiving the same TMR plus 10 g of synbiotic (Rumilax, EKO, Brazil); and Treatment 15 g (T15), receiving the same TMR plus 15 g of synbiotic. Dry matter intake (DMI) was monitored using automated feeders (Intergado, Minas Gerais, Brazil), and milk yield was recorded by the ALPRO electronic milking system (DeLaval, Tumba, Sweden). Feed efficiency was calculated as the ratio of milk yield to DMI. Statistical analysis was performed using the PROC MIXED procedure in JMP Pro 17, considering group, sampling day, and their interaction, with significance set at $P < 0.05$. The T15 group had lower DMI (20.61 ± 0.25 kg/day) compared with T10 and CON (22.89 ± 0.22 kg/day and 22.66 ± 0.27 kg/day, respectively, $P < 0.001$). Average milk yield did not differ among groups (T15: 39.63 ± 0.38 kg/day; T10: 39.87 ± 0.35 kg/day; CON: 39.68 ± 0.43 kg/day, $P = 0.88$). However, feed efficiency was higher in T15 (1.96 ± 0.02) compared with T10 and CON (1.73 ± 0.02 and 1.82 ± 0.02 , respectively, $P < 0.001$). In conclusion, supplementation with 15 g of the synbiotic improved feed efficiency, demonstrating its potential to enhance the productive performance of multiparous cows at peak lactation.

Key Words: gut health, peak lactation, animal nutrition

2453 Meta-analysis of the effects of replacing sulfate with hydroxychloride trace minerals on production performance in dairy cows. J. B. Cardoso*, W. P. Weiss², J. B. Daniel³, H. Dann⁴, T. R. Overton⁵, K. J. Harvatine⁶, J. Metcalf⁷, K. Perryman⁷, K. E. Griswold⁷, and J. E. P. Santos¹, ¹University of Florida, Gainesville, FL, ²Ohio State University, Columbus, OH, ³Trouw Nutrition R&D, Boxmeer, the Netherlands, ⁴Miner Institute, Chazy, NY, ⁵Cornell University, Ithaca, NY, ⁶Pennsylvania State University, State College, PA, ⁷Selko USA, Indianapolis, IN.

Table 1 (Abstr. 2453).

Item	Treatment		SEM	Difference	SED	P-value
	STM	HTM				
DMI, kg/d	24.6	24.8	0.9	0.24	0.27	0.38
Milk, kg/d	40.7	41.6	2.7	0.85	0.55	0.12
ECM, kg/d	40.9	41.9	1.9	0.97	0.55	0.07
ECM/DMI, kg/kg	1.68	1.71	0.05	0.025	0.019	0.18
Fat, %	3.73	3.75	0.21	0.019	0.044	0.66
Fat, kg/d	1.48	1.52	0.07	0.038	0.022	0.09
Protein, %	3.07	3.08	0.15	0.006	0.024	0.82
Protein, kg/d	1.22	1.25	0.05	0.029	0.016	0.08
SCS	2.73	2.74	0.54	0.009	0.151	0.95
BW, kg	646	650	12	3.9	7.1	0.58
NEL balance, Mcal/d	0.37	0.07	0.36	-0.29	0.36	0.42

A systematic review of the literature was conducted to identify randomized experiments in which supplemental Zn, Cu, and Mn from sulfate sources (STM) were replaced by hydroxychloride sources (HTM) in diets fed to dairy cows. The objectives were to determine effects of replacing STM with HTM on production performance in dairy cows. A total of 7 experiments fit the inclusion criteria, 5 of which were change-over design (CO; $n = 341$ experimental units) and 2 were continuous design (CONT; $n = 177$ experimental units). The NASEM dairy software (2021) was used to estimate the NEL content of diets used to calculate NEL balance. Individual cow Zn, Cu, and Mn intakes were calculated. Data were analyzed by mixed-effects models with the fixed effects of treatment (STM vs. HTM), experimental design (CO vs. CONT), and the interaction between treatment and design, and the random effect of experiment (1 to 7) nested within experimental design. Daily intakes of Zn (2,197 vs. $2,222 \pm 236$ mg/d) and Cu (488 vs. 491 ± 29 mg/d) did not differ between treatments, but cows fed STM had smaller ($P < 0.01$) intake of Mn compared with those fed HTM ($1,720$ vs. $1,814 \pm 72$ mg/d). The interaction between treatment and experimental design did not affect any of the responses analyzed, which are presented in Table 1. Feeding HTM tended ($P \leq 0.09$) to increase yields of ECM (1 kg/d), fat (38 g/d), and protein (29 g/d) without affecting DMI, BW, or NEL balance of cows. Replacing STM with HTM sources of Zn, Cu, and Mn benefited production performance in dairy cows.

Key Words: hydroxychloride, mineral, sulfate

2454 Effect of essential oils when supplied in feed or via a slow-release rumen bolus on plasma ketones and hepatic triglyceride concentration of early-lactation dairy cows. C. E. M. Roxborough^{*1}, A. M. Mackenzie¹, J. Bennison², and L. A. Sinclair¹, ¹Harper Adams University, Newport, Shropshire, UK, ²Agrimis Ltd., Kirmington, Lincolnshire, UK.

Essential oils are volatile compounds consisting of plant secondary metabolites that can beneficially alter rumen fermentation, potentially reducing the risk of ketosis and hepatic lipidosis. A slow-release rumen bolus is a practical, controlled means to administer essential oils, particularly for extensively grazed ruminants. The objective of this study was to examine the effect of essential oils supplied in a slow-release rumen bolus or in the diet on plasma ketone and hepatic triglyceride (TG) concentrations of early lactation dairy cows. Forty-five multiparous dairy cows that were (mean $\pm$ SE) 11 ± 0.6 d post calving and yielding 38 ± 0.9 kg milk/d were randomly allocated to one of 3 treatments: C: basal TMR, TO: basal TMR + essential oils (Agolin, Switzerland fed at 0.92 g/cow/d), or BO: basal TMR + intraruminal boluses containing

essential oils to provide the same daily rate of release as TO. Individual intake was recorded daily and plasma samples were collected every 2 wk for 10 wk. Hepatic samples were obtained via liver biopsy at wk 6 of the study. The data were analyzed as a mixed effect model, with time, treatment, lactation number, days postcalving, previous 305-d lactation yield and wk 0 data as fixed effects. There was no effect of treatment ($P = 0.124$ and $P = 0.581$, respectively) on plasma concentrations of BHB or nonesterified fatty acids, with means of 0.466 and 0.178 mmol/L, respectively. There was a lack of effect ($P = 0.284$) of treatment on hepatic TG concentrations, with a mean of 1.45 g/100 g. However, chi-squared analysis revealed a higher ($P < 0.05$) proportion of cows on treatment TO had TG concentrations $<1\%$ compared with those on C, with BO being intermediate. In conclusion, supplying essential oils in the TMR or via a slow-release rumen bolus had no effect on plasma metabolites in early lactation dairy cows. However, when added to the TMR, essential oils increased the proportion of cows with hepatic TG concentrations $<1\%$.

Key Words: essential oil, bolus, ketosis

2455 A quick method to measure non-carbon dioxide gas from a mixture of fermentation gases. A. Tiwari^{*} and R. Kohn, *University of Maryland, College Park, MD.*

Gas measurement in ruminal fermentation studies is one of the major challenges due to the high cost, time consumption, and requirement for highly skilled personnel to operate advanced gas chromatography equipment. Developing a reliable method for gas analysis is essential, especially for resource-limited research facilities in developing countries. Therefore, the objective of this study was to test the shaker method, developed in our laboratory, to measure the non-carbon dioxide portion of the gas mixture. Wheaton bottles ($n = 12$, 120 mL each) were initially filled with 15 mL of water and 10 mL of 6N NaOH solution was added. The bottles were plugged with rubber stoppers and sealed with aluminum caps. Gas mixtures of CO₂ and air in different ratios 4:0 (12:0 mL), 3:1 (9:3 mL), 2:2 (6:6 mL), and 1:3 (3:9 mL) were perfused into the bottles. After perfusion, the bottles were shaken by hand for at least 30 s. The final gas volume was measured using a glass syringe after the stopper popped up. The collected final gas volumes were plotted in graphs, showing a linear relationship between CO₂ disappearance and its increasing ratio in the mixture ($R^2 = 0.999$). The removal of CO₂ from the gas mixture allows for the estimation of the remaining non-carbon dioxide portion. Alternatively, CO₂ can be estimated by effects of pH buffering. Similarly, because H₂ gas occurs in very low concentrations during ruminal fermentation, its concentration can be estimated using

the Nernst equation and pH and Rh measurements. Future studies will compare the technique with measurements in rumen fluid with gas chromatography.

Key Words: gas measurement, gas mixture, methane

2456 Measuring the impact of within- and between-dairy variation when calculating a cluster randomized trial sample size. P. M. Lucey, T. L. Harris, and E. A. Horst*, *Elanco Animal Health, Greenfield, IN*.

Field demonstrations are cluster randomized trials where dairies are enrolled into treatment or control groups. These are used to assess feed additives because they may reflect “real-world” scenarios and offer statistical merit. However, dairy-to-dairy variability can limit statistical power and validity. This study quantified within- and between-dairy variation in milk yield per cow per day to determine sample sizes needed to detect differences of 1, 2, and 4.5 kg of milk. Data from 5 dairies across the United States with herd sizes of 2,800–10,000 lactating cows and including milk yield per cow (25–37 kg/d), and components (fat 3.7%–5% and protein 3%–3.8%), were collected from January 2019 to 2023; 4 dairies used Holstein and 1 used Jersey cows. A mixed model of milk per cow per day was built via stepwise selection, including a random effect of dairy, fixed effects of season in year, lactating head count and 2 spline terms for the fat-to-protein ratio to account for nonlinearity. Between- and within-dairy variances were 33 and 13 kg², with a marginal R² of 0.19 (fixed effects), a conditional R² of 0.77 (fixed and random), and an intraclass correlation coefficient (ICC) of 0.72. A sample size equation, using ICC, mean cluster size (lactating head count), and cluster size coefficient of variance (to account for uneven herd sizes), with $\alpha = 0.05$ and power = 0.80, estimated 764 (544–1,064), 123 (87–171), and 31 (22–43) dairies per treatment for detecting 1, 2, and 4.5 kg differences, respectively, after $\pm 20\%$ sensitivity analysis on ICC and cluster variance. A leave-one-out analysis removed the Jersey dairy for contributing the highest random error, producing new variances of 18.5 kg² and 13.4 kg² (ICC 0.58), marginal R² of 0.39, and conditional R² of 0.74; sample sizes dropped to 455 (336–599), 73 (54–96), and 19 (14–24) dairies. To power cluster randomized feed additive trials, investigators should minimize between-dairy variance such as breed, herd size, and location, and enroll sufficient dairies per treatment.

Key Words: field demonstration, cluster randomized trial, dairy variation

2457 Dietary liquid phosphorus binder decreases circulating phosphorus levels in dry cows. K. Gallagher*, S. Hagerty², and L. Rodriguez¹, *¹Innovative Liquids, LLC, Austin, TX, ²PHD R&D, Fort Atkinson, WI*.

Limiting dietary phosphorus (P) with phosphorus binders has been shown to limit circulating P levels and increase circulating calcium (Ca) in dry cows. Objective was to evaluate the response of a liquid dietary phosphorus binder with anionic capacity (iLimate, Innovative Liquids LLC) on dry cow DMI and serum markers. Multiparous Holstein cows blocked by gestation, parity, previous 305ME milk yield ($n = 18$; 219 ± 5 d gestation; 2.0 ± 0.8 parity; $3,5409 \pm 4,046$ 305ME; $X \pm SD$) received a top-dress of 1 of 3 treatments: ANI: dry anionic product (Phibro Animal Health Corp.) to meet urine pH = 6.0; LO IL: liquid P binder to meet TMR P:Al = 1.4; HI IL: liquid P binder to meet TMR P:Al = 1.15. Two 21-d periods were completed in a tiestall (7 d acclimation, 14 d treatment). Collections included daily cow DMI and rumination, activity, urine pH ($d = 0, 3, 6, 9, 12, 14$), serum and whole

blood ($d = 0, 6, 9, 12, 14$). Composition analysis of TMR was completed weekly on a basal diet (56% DM basis: 42.7% NDF, 12.1% CP, 19.5% starch, 0.58% Ca, 0.25% P). Treatment diets were balanced to a TMR DCAD of ANI = -15, LO IL = -6, HI IL = -11 mEq/100 g of DM. Mixed statistical model included random effect of cow within block by treatment, fixed effects of treatment, block, time, and treatment by time interaction. No treatment effect on DMI but a day effect (cubic, $P < 0.01$) was observed. Urine pH decreased with ANI ($P < 0.01$; ANI = 6.2, LO IL = 7.6, HI IL = 7.2). Time spent eating, ruminating, and chewing had no treatment effect. Serum Ca increased over time (linear effect, $P < 0.01$) with tendency to increase HI IL relative to ANI (9.8 vs. 9.4 mg/dL; $P = 0.06$). Serum P levels decreased on LO IL and HI IL relative to ANI (4.1 vs. 4.4 vs. 5.8 mg/dL; $P < 0.01$). Serum chloride had a COV effect ($P < 0.01$) but increased on ANI relative to LO IL or HI IL ($P = 0.03$; 101.8, 100.6, 100.1 mEq/dL), while serum sodium, magnesium, potassium, glucose, BHBA, and BUN did not differ between treatments. In summary, a liquid dietary phosphorus binder mildly acidifying dry cows on a close-up ration decreased serum P while increasing serum Ca ($P < 0.06$) compared with a more negative DCAD strategy without P binding capacity.

Key Words: phosphorus binder, calcium, dry cow

2458 Effects of rumen-protected choline during the pre- and postpartum periods on the metabolism and health of dairy cows. M. Bugoni*, L. S. Nogueira¹, A. C. Mocelin¹, C. S. Takiya², J. H. Carneiro³, J. O. Goelema⁴, J. B. Veneman⁴, and R. Almeida¹, *¹Universidade Federal do Paraná, Curitiba, PR, Brazil, ²Universidade Tecnológica Federal do Paraná, Pato Branco, PR, Brazil, ³Dairy Inside Consultoria, Castro, PR, Brazil, ⁴De Heus Animal Nutrition, Ede, the Netherlands*.

The objective was to evaluate the effect of rumen-protected choline (RPC) supplementation on the metabolism and health of transition dairy cows. In a commercial herd in Paraná State, Southern Brazil, 211 pregnant Holsteins (68 heifers and 143 cows) were blocked according to parity and expected calving date and were enrolled in a randomized block design experiment with a 2×2 factorial treatment arrangement. The RPC supplementation (30 g/d or 13.4 g/d of choline ion; Reashure XC, Balchem) was offered top-dressed onto TMR from 28 ± 5 d before until 26 ± 6 d after calving. Treatments included: (1) CON: control, no RPC; (2) CPre: RPC fed only during the prepartum; (3) CPost: RPC fed only during the postpartum; (4) CBoth: RPC fed during the pre- and postpartum periods. Blood metabolites were analyzed using the MIXED procedure of SAS, modeling the fixed effects of pre- and postpartum supplementation, parity, sampling day ($d -14, -7, 0, 7$, and 14), and their interactions. Non-normal variables were numerically transformed. Block and cow were considered random effects. Disease incidence variables were analyzed using the GLIMMIX procedure, including the fixed effects of pre- and postpartum supplementation, their interaction, and the random effect of block. An interaction between RPC pre- and postpartum supplementation was observed for BHB (0.82, 0.84, 0.86, and 0.77 mmol/L; $P = 0.04$ for CON, CPre, CPost, and CBoth, respectively). A tendency for interaction was also detected for total calcium and triglycerides (2.17, 2.22, 2.20, and 2.20 mmol/L; $P = 0.08$, and 8.54, 8.00, 8.06, and 8.36 mg/dL; $P = 0.06$ for CON, CPre, CPost, and CBoth, respectively). The GGT and AST enzymes, cholesterol, glucose, total protein, albumin, bilirubin, or NEFA concentrations did not differ ($P > 0.10$) among treatments. A tendency for an interaction was observed for the incidence of metritis (9.1%, 1.8%, 1.9%, and 5.5%, for CON, CPre, CPost, and CPre and Post, respectively; $P = 0.08$). Treatment did not influence ($P > 0.10$) the incidence of retained placenta, ketosis, mastitis,

or displaced abomasum. Metabolism and health were modestly affected by pre- or postpartum RPC supplementation.

Key Words: choline, disease, transition period

2459 Supplementation of rumen-protected B vitamin blend to dairy cattle during the transition period. S. Dean^{*1}, V. Asselstine¹, G. Mechor¹, E. Evans², and E. Fontaine¹, ¹*Jebo Nutrition Inc., St. Hyacinthe, QC, Canada*, ²*Technical Advisory Services, Bowmanville, ON, Canada*.

During the transition period, dairy cows experience physiological, metabolic, and immunological changes, increasing their susceptibility to diseases. Nutritional strategies during this period can support metabolic health. This field study aimed to evaluate the supplementation of a rumen protected B vitamin blend (RPBV; B9, B2, B12, and choline; 100 g/head per day) compared with a control (protected choline only; 60 g/head per day) during the prepartum transition period (21 d) of dairy cattle. This trial compared 3 periods, Period 1: January 2023–August 2023, Period 2: September 2023–February 2024, Period 3: February 2024–May 2024. Periods 1 and 3 were combined (517 calvings) and compared with Period 2 (457 calvings). During all periods, cows received a basal corn silage and haylage-based TMR diet, with RPBV supplementation beginning 3 weeks before parturition in Period 2. Cow performance was evaluated using data from 3 monthly milk tests, including only cows with data from all 3 test dates. Treatments were compared using a general linear model. Period 1 + 3 vs. Period 2 results for all milk related parameters were compared by cow as a randomized complete block. Test cows showed increased ECM at the third test date (+2.3 kg; Table 1). Health records were evaluated for the first 30 DIM. Fisher’s exact test was used to determine differences in incidence rates. The RPBV cows had decreased prevalence of retained placenta, ketosis <15 DIM, and pneumonia and mastitis <30 DIM (Table 1). Results indicated that RPBV cows had a lower removal rate from the herd before first breeding (–8.21%) and confirmed pregnancy (–3.44%). Results from this trial demonstrate that a blend of B vitamins and choline provides greater benefits than choline supplementation alone. Future research to investigate the underlying mechanisms for the response obtained is needed.

Key Words: B vitamin, transition, health

2460 Effects of replacement of multi-anionic salts with a single coated calcium chloride source on performance, health, and metabolic status of periparturient cows. A. Jan¹, A. Hassouna², N. Ali Sahar², D. Martinez Del Olmo¹, M. Al Rifai¹, and E. E. Gültepe^{*1}, ¹*Kemin Europa NV, Herentals, Belgium*, ²*Umer Farms, Lahore, Pakistan*.

This study evaluated the replacement of 3 anionic salts in close-up diets with a single encapsulated calcium chloride source (enCaCl). Multiparous cows were randomly assigned to receive either enCaCl (270 g/

head per day, n = 74; NutriCAB, Kemin Animal Nutrition and Health, Belgium; DCAB –192 mEq/kg DM) or multi-anionic salts (mAS; 320 g/head per day, n = 66; 100 g CaCl₂, 120 g MgSO₄, and 100 g NH₄Cl; DCAB –252 mEq/kg DM) from 28 d prepartum until calving. After a common far-off diet, cows were housed in separate close-up pens, moved to lactation pens post-calving, and milked twice daily. Blood and spot urine samples were collected once per animal during the close-up period, with urine pH monitored for net acid excretion. Production data were extracted from the dairy system. Colostrum samples were also analyzed. Data quality was ensured via robust fit outlier detection (Huber M-Estimation, K = 4), with log-transformation as needed. A mixed model (REML, JMP v18) was used, considering treatment as a fixed effect and cow as a random effect. LSmeans were reported, with significance at $P < 0.05$. Serum tCa (11.3 vs. 10.2 ± 0.4 mg/dL), P (6.4 vs. 5.9 ± 0.2 mg/dL), Ca:P ratio (1.8 vs. 1.6 ± 0.1), and urine pH (5.82 vs. 5.96 ± 0.09) were similar between mAS and enCaCl groups ($P > 0.05$). However, Mg was significantly higher in enCaCl cows (0.9 vs. 2.2 ± 0.3 mg/dL, $P = 0.002$). After the 3-d colostrum period, milk yield on d 1 (38.5 vs. 41.4 ± 1.2 kg), d 2 (48.0 vs. 48.9 ± 0.8 kg), and peak milk yield (48.5 vs. 49.7 ± 0.7 kg) tended to be higher ($P > 0.05$) in enCaCl compared with mAS cows. Colostral fat, SNF, total solids, protein, lactose, Brix, and pH were comparable ($P > 0.05$). These findings suggest that replacing multi-anionic sources with a single encapsulated CaCl₂ product can support anionic nutrition without adverse prepartum effects.

Key Words: encapsulation, calcium, DCAD

2461 The impact of free-choice bicarbonate/trace mineral consumption on performance of Holstein cows at varying stages of lactation during the summer in northern New York. R. Matsuda², A. E. Pape¹, H. M. Gauthier¹, H. E. Jones¹, and C. S. Ballard^{*1}, ¹*William H. Miner Agricultural Research Institute, Chazy, NY*, ²*ZENOAG, Fukushima, Japan*.

A retrospective study was conducted to investigate performance effects of free-choice bicarbonate/trace mineral block use on early (E), mid (M) and late (L) lactation Holstein cows. Initially, 45 multiparous cows equally representing 3 stages of lactation: E = 84 ± 5 DIM, M = 146 ± 22 DIM, and L = 208 ± 20 DIM were enrolled in August 2024. Cows were housed in a sand-bedded freestall, milked 3 0215×/d and pen-fed a basal TMR 1×/d balanced with buffer sources for 30 kg/d DMI and 51.7 kg/d milk. After a 1 wk covariate period, 4 low-moisture, bicarbonate/trace mineral blocks were placed in holders mounted in 4 locations by waterers for 4 weeks. Blocks were weighed daily, and consumption measured on a pen basis. Individual cows were observed for time spent licking blocks via video daily (24 h) and summarized in min/d for the 4 wk blocks were available. Cows were classified by overall mean time consuming blocks: Low = <25 min/d (n = 15); Med = 25–50 min/d (n = 20); and High = >50 min/d (n = 8). Daily milk yields and milk composition analyzed from 6 consecutive milkings weekly were reduced to weekly means. Data were subjected to analysis of covariance using the MIXED procedure of SAS (v. 9.4) with the model including covariate and fixed effects of block consumption (USE), stage of lactation (STAGE), USE × STAGE, and week. Cow was random effect, and week as repeated measures. Least squares means were separated using Tukey’s procedure when significant F-test ($P \leq 0.05$) was detected. Forty-three cows finished the study and collectively consumed 10.2 ± 1.4 kg of blocks/d. Milk yield was lowest for cows that consumed blocks <25 min/d, with early lactation animals that consumed the block >25 min/d appearing to benefit the most, averaging 4.39 kg/d more milk than early lactation cows consuming blocks <25 min/d. Milk fat and milk protein were not influenced by block consumption. The combined effects of

Table 1 (Abstr. 2459). Evaluation of milk parameters and health parameters

Item	Period 1+3	Period 2	P-value
ECM 3rd test, kg	51.0	53.3	<0.001
Retained placenta %	11.27	5.28	<0.001
Ketosis %, <15 DIM	5.48	2.85	0.038
Pneumonia %, <30 DIM	8.29	3.86	0.003
Mastitis %, <30 DIM	1.88	0.41	0.030

Table 1 (Abstr. 2461). Effect of bicarbonate/trace mineral block use on performance of lactating dairy cows

Item	Use group			SEM	P-value		
	Low	Med	High		Use	Stage	Use × stage
n	15	20	8	—	—	—	—
Milk, kg/d	46.2 ^b	48.0 ^a	47.6 ^{ab}	0.50	0.03	0.01	0.02
Milk fat, %	4.07	4.03	3.97	0.05	0.42	0.07	0.91
Milk protein, %	3.14	3.16	3.12	0.01	0.37	0.87	0.16

^{a,b}Within rows, different superscripts are significantly different, $P < 0.05$.

licking, which stimulates saliva production, and block ingredients may support rumen health in high producing cows. Results of the experiment are shown in Table 1.

Key Words: bicarbonate, trace mineral, dairy cow

2462 Effects of a liquid negative DCAD supplement on transition cow performance. B. Juárez^{*1}, T. Forgues¹, A. Mueller², and A. J. Carpenter¹, ¹Iowa State University, Ames, IA, ²Westway Feed Products, The Woodlands, TX.

The objective of this study was to determine the effects of feeding a liquid molasses-based negative DCAD supplement (RumaWAY LAUNCH, Westway Feed Products, The Woodlands, TX) on pre- and post-calving DMI, metabolism, and production. Multiparous Holstein cows ($n = 48$) were blocked by parity, DIM at dry-off, and projected 305-d actual milk, and assigned to one of 2 diets: a TMR diet containing the molasses-based supplement (MOL) and a TMR containing a commercial negative DCAD mineral (CON). Cows were enrolled 28 d before expected calving. At calving, cows were switched to a common lactation diet through 60 DIM. Their DMI was measured daily using Calan feeders during the pre- and post-calving period. Calcium, nonesterified fatty acids (NEFA), and BHB were collected on d -10, -7, 1, 3, 7, 10, 14, 21, and 28 relative to calving along with BCS. Milk weights were collected daily, and milk samples were collected weekly for component analysis. Colostrum weight was recorded, and a subsample was collected to determine Brix scores. Urine pH was measured weekly. Data were analyzed using PROC MIXED in SAS 9.4 with the effects of diet, parity, time, diet × parity, and diet × time. Precalving DMI varied by week ($P < 0.01$), while post-calving DMI tended to show a diet × week interaction ($P = 0.07$). Dietary treatment, parity, or their interactions did not significantly impact blood metabolites pre- and post-calving. However, post-calving BHB was influenced by parity ($P = 0.05$), with a tendency for differences in calcium on d 3. Milk yield and components were not impacted by diet ($P \geq 0.27$). Colostrum Brix did not differ between treatments, while yield had a tendency for diet × parity interaction ($P = 0.07$). Urine pH showed a diet × week interaction ($P < 0.01$). Diet tended to affect BCS ($P = 0.06$) with both treatments maintaining a BCS of 3–3.25. These results suggest that supplementing a negative DCAD in liquid molasses precalving does not negatively influence transition cow health or production and may impact early lactation DMI.

Key Words: transition cow, performance, negative DCAD

2463 Dietary association of proteinate minerals and alpha-amylase on apparent absorption and retention of microminerals in dairy cows. R. G. Chesini¹, N. T. S. Grigoletto¹, C. S. Takiya^{1,2}, G. G. da Silva¹, D. J. C. Vieira¹, A. F. Koontz³, L. F. C. Silva³, and F. P. Rennó^{*1}, ¹University of São Paulo, Pirassununga, São Paulo, Brazil,

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Trace mineral (TM) sources may interact with enzymes in the gut of monogastric animals, but data on ruminants is limited. An experiment was conducted aiming to evaluate the apparent absorption (ABS) and retention (RET) of inorganic (INO) and proteinate trace minerals (PM) when fed with α -amylase (AMY). Eight ruminal cannulated Holstein cows (29.5 ± 3.66 kg/d milk yield, 216 ± 30 DIM) were blocked by parity and assigned to a replicated 4×4 Latin square (LS) experiment with 21-d periods. Cows were randomly assigned to the following treatments: (1) CON, diet with INO trace minerals (Co, Cu, and Mn as sulfates, Zn oxide, and Se as sodium selenite); (2) ENZ: CON treatment plus AMY (Amaize, Alltech) at 0.5 g/kg DM; (3) PM, diet with PM replacing INO sources (Co, Cu, Mn, and Zn as PM, and Se as Se-yeast; Bioplex and Sel-Plex, Alltech); or (4) PMA: PM treatment plus AMY. The TM concentrations followed the NASEM guidelines for all treatments. Cows were housed in a tiestall barn and fed individually. The TMR and orts were weighed and sampled daily to quantify TM intake. Total feces and urine collection were performed between d 17 and 20 of each period. Cows were milked twice daily, and milk samples were collected during 3 consecutive days for analysis. Samples of TMR, orts, feces, urine, and milk were analyzed for TM using wet chemistry. Data were analyzed by the Mixed procedure of SAS with the fixed effects of LS, period, inclusion of PM or AMY, their interaction, and the random effect of cow within LS. The ABS (%) of Zn (45.6 vs. 31.6; $P < 0.01$), Mn (32.0 vs. 10.9; $P < 0.01$), Cu (35.7 vs. 24.0; $P = 0.03$), Co (39.1 vs. 29.5; $P < 0.01$), and Se (47.6 vs. 38.3; $P < 0.01$) were higher for PM than INO diets. Similarly, higher RET (%) was observed for PM diets compared with INO for Zn (37.9 vs. 23.7; $P < 0.01$), Mn (31.9 vs. 10.8; $P < 0.01$), Cu (35.6 vs. 24.1; $P = 0.03$), Co (29.3 vs. 21.4; $P = 0.02$), and Se (23.6 vs. 8.45; $P < 0.01$). There was no significant difference with the addition of enzyme to diets or interaction between ENZ and minerals sources on ABS or RET. Feeding PM benefited apparent absorption and retention of trace minerals in dairy cows.

Key Words: enzyme, organic mineral

2464 Performance of dairy cows fed a combination of essential oils and live yeast. N. P. Martins¹, R. G. Chesini¹, N. T. S. Grigoletto¹, B. C. Costa¹, A. J. C. Oliveira¹, N. T. Silva¹, G. Poletti¹, C. S. Takiya^{2,1}, T. Durman³, L. F. C. Silva³, and F. P. Rennó^{*1}, ¹University of São Paulo, Pirassununga, São Paulo, Brazil, ²Universidade Tecnológica Federal do Paraná, Pato Branco, Paraná, Brazil, ³Alltech do Brasil, Maringá, Paraná, Brazil.

Plant extracts and yeast products have been studied for their potential to improve gut health. An experiment was conducted aiming to evaluate nutrient digestibility, milk yield (MY), and milk composition in cows fed a blend of essential oils and live yeast. Forty-two Holstein cows (177 ± 56 DIM, 36.4 ± 5.26 kg/d MY) were blocked ($n = 14$) by parity, DIM, and MY and were assigned to one of the 3 treatments: (1) Control (CON), no feed additives; (2) Essential oils (EO), 1 g/cow per day of a proprietary blend of essential oils (Agolin Ruminant, Alltech); or (3) EO and live yeast (EOY), EO plus 2 g/cow per day of live yeast (5×10^9 cfu/g; Yea-Sacc, *Saccharomyces cerevisiae* strain 1026, Alltech). The experiment lasted 11 wk, with the first 2 wk for covariate data collection. Treatments were incorporated into the concentrate mix, and cows were individually fed a TMR (48:52 forage-to-concentrate ratio). The TMR, refusals, and feces were analyzed for chemical composition to determine nutrient intake and excretion. Fecal samples were collected over

3 consecutive days in wk 3, 6, and 9, and pooled by week for analysis. Daily fecal excretion was estimated using indigestible NDF as internal marker. Cows were milked twice daily, and milk samples were collected over 6 consecutive milkings weekly. Milk samples were pooled by day for analysis. Data were analyzed using the MIXED procedure of SAS, with fixed effects of covariate, treatment, time, and their interactions, and block as a random effect. Orthogonal contrasts were used to verify treatment differences (CON vs. EO+EOY and EO vs. EOY). The DM, NDF, and CP intake increased ($P \leq 0.05$) with the use of feed additives. Apparent total-tract OM digestibility tended to be higher ($P = 0.09$) in EOY compared with EO (69.4% vs. 68.4%). Milk yield was greater ($P < 0.01$) with feed additives (35.2, 37.2, and 36.4 kg/d for CON, EO, and EOY, respectively) as were the 3.5% FCM and ECM yields ($P < 0.01$). Fat and protein yields were higher ($P \leq 0.01$) in additive-fed cows. Feed efficiency tended to be greater ($P = 0.06$) in EOY than EO (1.39 vs. 1.34 kg/k). Feeding a combination of EO and live yeasts improved milk production without negatively affecting nutrient digestibility.

Key Words: additive, efficiency

2465 Effects of NPN supplementation on lactating Holstein cows. G. Ponce¹, M. R. R. Nair¹, I. R. Ipharraguerre², I. Ruiz-Ascacibar³, and A. G. Rius^{*1}, ¹University of Tennessee, Knoxville, TN, ²University of Kiel, Kiel, Germany, ³Yara Sluiskil, Sluiskil, the Netherlands.

The objective of this study was to assess the effect of an NPN source consisting of a urea-biuret mixture (UB) on performance of high-producing dairy cows on corn-based rations. The study was conducted as a complete randomized design at East Tennessee Research and Teaching Center – Little River Animal and Environmental Unit in 2024. Forty-eight Holstein cows (parity 1, 2 and ≥ 3 ; 44 ± 7.8 kg milk/d) were stratified by parity, milk yield, and days in milk, and randomly allocated to (1) control (CTL), (2) urea-biuret level 1 (UB1), and (3) urea-biuret level 2 (UB2) dietary treatment for 12 wk ($n = 16$ per treatment). The diets contained 131 g urea/cow per day for the CTL cows, 137 g UB/cow per day for the UB1 cows, and 237 g UB per/cow per day for the UB2 cows. The treatments were designed according to industry recommendations using NASEM (2021) model to be isoenergetic (1.80 Mcal/kg of NEL) and provide 17.0% CP (CTL and UB1) and 18.0% CP (UB2). Dietary treatments included ingredients typically used in commercial operations in the Southeast such as corn silage, straw, corn grain, soybean meal, protected soybean meal, soybean hulls, rumen-protected fat, and a premix of minerals and vitamins. Treatments were fed twice daily to achieve ad libitum intake (5%–10% refusals) using an automatic feeding system. The dry matter concentration of forage ingredients was determined, and diets were adjusted weekly. Feed ingredients and diets were analyzed by a commercial laboratory. Milk samples were collected twice a week. The statistical analysis was performed using mixed models in SAS, and data collected before treatment initiation was included as a covariate. Individual cows were used as the experimental unit for all dependent variables. The model included the fixed effects of treatment, time, and parity and the random effect of cow. Data were analyzed as repeated measures in time and the most suitable covariance structure was chosen using the smallest AIC values. The dry matter intake was not affected by treatments. There was a treatment $\times$ parity interaction ($P < 0.01$), and milk yield increased in UB1 compared with CTL in parity ≥ 3 cows ($P < 0.01$). The milk fat concentration and yield increased in UB2 compared with CTL and UB1 ($P < 0.01$). Milk protein yield decreased in UB1 compared with CTL and UB2 ($P < 0.01$). Compared with CTL and UB1, UB2 increased MUN ($P < 0.01$). Results indicate that the NPN product supports the productivity of dairy cows fed corn-based diets.

Key Words: biuret, dairy cattle, nitrogen

2466 Effect of dietary choline and diet fermentability on postpartum dairy cow performance and feeding behavior. K. R. Pasch*, F. Vignati, and W. E. Brown, *Kansas State University, Manhattan, KS.*

Highly fermentable diets depress dry matter intake (DMI) in postpartum dairy cows due to rapid oxidation of acetyl CoA after meals. Postpartum cows have elevated body fat mobilization and hepatic lipid accumulation, increasing hepatic acetyl CoA. Rumen-protected choline (RPC) may reduce acetyl CoA available for oxidation through hepatic lipid export. Our objective was to assess the effect of diet fermentability (DF) and RPC supplementation on postpartum cow DMI and performance. Multiparous Holstein cows ($n = 65$) were enrolled 21 d prepartum and supplemented with RPC (C+; 30 g/d; ReaShure, Balchem) or no RPC (C–) and fed with a computerized feeding system (RIC; Hokofarm). The postpartum study was a randomized complete block design with a 2×2 factorial treatment (trt) arrangement: RPC (C– or C+) and starch fermentability rate (low [dry rolled corn; LFERM] vs. high [wheat; HFERM]). Postpartum cows ($n = 56$) were fed for 21 d postpartum in a tiestall with a computerized feeding behavior system. Feeding behaviors, DMI, and milk yield were collected daily, and milk components 2 d/wk. Data were analyzed for trt, time, and their interactions using PROC MIXED in SAS (v 9.4). Means are presented with 95% CI. Prepartum, C+ decreased DMI compared with C– ($P < 0.01$; 14.8 [13.8,15.9] vs. 17.1 kg [16.0,18.2]). Postpartum, there was no evidence of trt difference on DMI ($P > 0.19$). Time and RPC interacted on meal size ($P = 0.04$) with C– increasing meal size more rapidly than C+ through d 10 and after d 15. Time and DF interacted on milk yield ($P = 0.02$) with HFERM increasing milk yield after d 3 compared with LFERM. High fermentability treatment decreased milk fat % ($P = 0.01$; 4.4 [4.0,4.8] vs. 4.8% [4.4,5.2]) but not fat yield ($P = 0.16$). Time and DF interacted on protein % ($P = 0.04$) and yield ($P = 0.05$); HFERM decreased protein yield from wk 1 to 3 (3.1 [2.8,3.4] vs. 2.8 [2.6,3.1]), but there was no difference across week for LFERM. There were no interactions between RPC and DF ($P > 0.10$). Overall, feeding a highly fermentable diet decreased milk fat % and increased milk yield over time. Dietary RPC depressed DMI prepartum, but not postpartum.

Key Words: transition period, feeding behavior

2467 Prepartum administration of *Macleaya cordata* extract bolus and its effects on dairy cows' postpartum health, milk production, and somatic cell score. L. S. Nogueira^{*1}, H. P. Janssen², R. M. T. Silva³, and R. Almeida¹, ¹Universidade Federal do Paraná, Curitiba, PR, Brazil, ²Castrolanda Cooperativa Agroindustrial, Castro, PR, Brazil, ³Phytobiotics Brasil, Cambé, PR, Brazil.

The aim of this study was to investigate the association between prepartum administration of *Macleaya cordata* extract (MCE) to dairy cows and the effects on milk production, SCS, and health. A total of 129 multiparous Holstein cows (BW: 717.8 ± 56.3 kg; BCS: 3.13 ± 0.28) from a commercial herd located in Paraná State, Southern Brazil, were blocked randomly based on parity and expected calving date. Cows were divided in 3 treatment groups based on the application or not of MCE bolus (Bolus Lacto V6, Phytobiotics, Germany). Twenty-four days before expected calving date, cows received either one bolus of 75 g (B1; equivalent to a dose of 1.5 g/d MCE), 2 boluses of 75 g (B2; equivalent to a dose of 3.0 g/d MCE), or no MCE bolus (CON). Individual milk samples were collected weekly during experimental period. Blood samples were collected at calving, +3, +7, +14, and +28 d postpartum. Data were analyzed through MIXED procedure of SAS containing the fixed effects of treatment, time, and their interactions, and the random effects of block and cow within treatment. Milk production was not affected by MCE administration (52.90, 52.22, and 52.60 kg/d

for CON, B1, and B2, respectively; $P = 0.88$). In addition, no changes ($P > 0.10$) were observed in milk composition. There was a tendency of lower linear SCS for MCE-treated animals (2.69, 2.55, and 2.55, respectively; $P < 0.10$). Serum GGT concentrations were higher ($P < 0.01$) for the CON group at d 7 and 14 postpartum, while AST levels were elevated ($P < 0.01$) at d 7, 14, and 28, suggesting a protective effect of MCE against liver oxidative damage. Although it did not significantly affect milk production, MCE prepartum administration affected udder and liver health by reducing milk SCS and GGT and AST serum concentrations, indicating that it contributes to health status improvement during the transition period.

Key Words: hepatic enzyme, herbal extract, transition period

2468 Supplemental magnesium sources display a wide range of solubility consistently across methods. B. A. Wenner^{*1}, A. H. Weston², W. B. Fokkink², J. Travis³, S. Ranathunga³, S. O'Connell⁴, B. Naughton⁴, O. Demehin⁴, and J. M. Aldrich², ¹Feedworks USA, Cincinnati, OH, ²CSA Animal Nutrition, Dayton, OH, ³Cumberland Valley Analytical Services, Waynesboro, PA, ⁴Marigot Research Centre, Tralee, Co. Kerry, Ireland.

Adequate bioavailable Mg is crucial for dairy cows. However, high Mg content in supplements does not ensure high bioavailability, which depends on solubility, reactivity with other rumen solutes, and absorption. Although Mg absorbability varies by source, MgO commonly has a fixed absorption coefficient (AC) in feed libraries. The objective of this study was to quantify the variability of Mg solubility and compare estimation methods for AC to screen for supplemental Mg source quality. In total, 19 supplemental Mg sources were analyzed for solubility by using 3 methods which were: (1) vinegar (strong acetic acid) and measuring pH after 30 min, then estimating solubility using a published meta-analysis equation (method 1); (2) published acid titration (0.1 M HCl) to a pH of 5.5 for 2 h and quantifying soluble Mg (method 2); and (3) novel acid titration with 1 M acetic acid to a pH 5.5 at 0, 2, 4, and 6 h, then quantifying soluble Mg at 6 h (method 3). At least 3 replicates were run for each method for each sample; averages were used to compare methods for estimation of AC. To account for the buffering effect of Ca carbonate in method 1, acid neutralization of 4 grades of Ca carbonate were used to correct for Ca concentration of samples compared within the Mg solubility equation ($-0.182 \times \%Ca$). Data from all 3 methods were analyzed using a linear model for general correlation and Kendall Tau test for ranking comparison performed within R (v. 4.4.0). The adjusted R^2 between the 3 methods were 0.54, 0.53, and 0.36 for Method 1 vs. 2, Method 1 vs. 3, and Method 2 vs. 3, respectively (all $P \leq 0.01$). Kendall Tau tests implied positive correlation of 0.40 ($P = 0.02$), 0.35 ($P = 0.07$), and 0.43 ($P = 0.03$) for ranking Mg sources by method 1 vs. 2, method 1 vs. 3, and method 2 vs. 3, respectively. Method 1 had good agreement with the other 2 methods and remains the simplest and most cost-effective tool to estimate Mg solubility. Method 1 Mg solubilities ranged from 0.09 to 0.79 and showed high variation in Mg solubility. Method 3 reported solubilities at every 2 h which could lead to development of Mg solubility curves with dataset expansion and process refinement.

Key Words: transition cow, calcium

2469 Effect of duration of feeding a rumen-protected choline prototype during the dry period and early lactation on transition cow performance. L. N. Ferro^{*1}, A. L. Kerwin¹, C. M. Horner¹, M. R. Arroyo¹, T. M. Nelson¹, G. M. Graef¹, C. Zimmerman², K. Estes², D. M. Barbano³, and T. R. Overton¹, ¹Department of Animal Science Cornell

University, Ithaca, NY, ²Balchem Corp., Montvale, NJ, ³Department of Food Science Cornell University, Ithaca, NY.

The objective was to determine performance effects of a rumen-protected choline prototype (RPCP; Balchem Corp.) for different feeding durations during the dry period and early lactation. In a randomized complete block design, Holstein cows entering ≥ 2 lactations were enrolled at dry-off (~ 10 wk prepartum) and assigned to one of 3 treatments 1 wk later: Control (CON; $n = 24$) = no RPCP; transition (TC; $n = 23$) = 30 g/d of RPCP from ~ 4 wk prepartum to 3 wk of lactation (WOL); full (FC; $n = 24$) = 30 g/d of RPCP from ~ 9 wk prepartum through 3 WOL. Cows moved to a freestall pen with Calan gates at dry-off until 4 wk prepartum, moved to tiestalls through 5 WOL, then moved to a freestall pen through 15 WOL. Intake (through 5 WOL) and milk yield were recorded daily and reduced to weekly means. Milk components, BW, and BCS were collected 1 $\times$ /wk. Continuous data were analyzed using PROC MIXED of SAS, with repeated measures if applicable, and categorical data were analyzed by Fisher's exact test using PROC FREQ. Prepartum and postpartum data were analyzed separately. There was no significant treatment effect for milk yield ($P \geq 0.87$), ECM (1 to 5 WOL CON vs. TC vs. FC: 51.6 vs. 54.2 vs. 53.0 $\pm$ 1.3 kg/d; $P = 0.34$; 1 to 15 WOL CON vs. TC vs. FC: 53.4 vs. 55.0 vs. 54.8 $\pm$ 1.1 kg/d; $P = 0.50$) or milk components ($P > 0.05$), for BW prepartum (CON vs. TC vs. FC: 788 vs. 791 vs. 792 $\pm$ 4 kg; $P = 0.79$), 1 to 5 WOL (CON vs. TC vs. FC: 704 vs. 712 vs. 714 $\pm$ 7 kg; $P = 0.56$), or 1 to 15 WOL (CON vs. TC vs. FC: 706 vs. 709 vs. 711 $\pm$ 6 kg; $P = 0.85$), for prepartum (CON vs. TC vs. FC: 14.8 vs. 15.1 vs. 14.7 $\pm$ 0.4 kg/d; $P = 0.71$) or postpartum DMI (CON vs. TC vs. FC: 23.2 vs. 23.6 vs. 23.9 $\pm$ 0.6 kg/d; $P = 0.67$), or for DMI as a percent of BW prepartum ($P = 0.44$) or postpartum (CON vs. TC vs. FC: 3.3 vs. 3.4 vs. 3.4 $\pm$ 0.1; $P = 0.62$). There was no difference in the distribution of BCS across treatments at wk -8, -1, 1, 5, or 15 relative to parturition ($P \geq 0.13$). Overall, neither supplementation of this particular RPCP nor feeding duration of RPCP affected performance measures compared with cows fed CON.

Key Words: rumen-protected choline, transition cow, performance

2470 Effect of delactosed whey permeate on lactation performance and total tract nutrient digestibility by dairy cows. K. A. Juckem^{*}, E. C. Diepersloot, M. R. Pupo, and L. F. Ferraretto, *Department of Animal and Dairy Sciences, University of Wisconsin-Madison, Madison, WI.*

The objective of this study was to evaluate the effects of forage fiber and starch sources replacement with delactosed whey permeate (DLP) on lactation performance and total-tract nutrient digestibility of high-producing dairy cows. Ninety-six multiparous Holstein cows (88 ± 36 DIM) and dietary treatments were randomly assigned to 12 pens of 8 cows for an 8-wk treatment period, after a 2-wk covariate period. Treatments were diets fed without DLP (CON), 5% replacement of corn silage with DLP (LCS), and 5% replacement of high-moisture corn with DLP (LHMC). Data were analyzed as a completely randomized design with data from the 2-wk covariate period used as a covariate using a mixed model with week of treatment as repeated measures. The model included treatment, week, and their interaction as fixed effects, and pen (treatment) as a random effect. The CON diet tended ($P < 0.10$) to have 1.4 kg/d greater DMI than LCS but did not differ from LHMC. The CON diet had a greater ($P < 0.05$) milk yield compared with LCS by 2.2 kg/d and LHMC by 2.7 kg/d. Similarly, cows fed CON had 0.04 percentage units and 0.07 percentage units greater ($P < 0.05$) milk lactose concentration compared with LHMC and LCS, respectively. Milk fat did not differ between treatments ($P > 0.10$). Diets with partial replacement with DLP had 0.07 percentage units greater ($P < 0.05$) milk protein concentration.

However, no effects on feed efficiency were observed ($P > 0.10$) with replacement with DLP. Digestibility of DM, OM, and CP were greater ($P < 0.05$) for LCS diet compared with LHMC by 3.4 percentage units, 3 percentage units, and 3.8 percentage units, respectively. Ether extract digestibility tended ($P < 0.10$) to be 4.1 percentage units greater for LCS than LHMC. Additionally, NDF digestibility of the LCS diet was greater ($P < 0.05$) than CON and LHMC by 6.1%, on average. These findings suggest that replacing high-moisture corn or corn silage with DLP may decrease milk yield and milk lactose concentration but increase milk protein concentration. However, replacing corn silage with DLP can have more pronounced effects on total-tract digestibility compared with a replacement of high-moisture corn with DLP.

Key Words: DLP, milk production, digestibility

2471 Impact of decreasing undigested neutral detergent fiber (uNDF) concentrations on feeding behavior and sorting index in dairy cows. N. T. Scognamiglio Grigoletto^{*1}, D. J. C. Vieira², R. G. Chesini¹, O. P. Sbaralho¹, M. S. R. Serrasqueiro¹, N. Terassi da Silva¹, C. S. Takiya³, and F. P. Rennó¹, ¹University of São Paulo, Pirassununga, SP, Brazil, ²University of Wisconsin–Madison, Madison, WI, ³Universidade Tecnológica Federal do Paraná, Pato Branco, PR, Brazil.

This study aimed to evaluate the impact of decreasing dietary uNDF concentrations on feeding behavior and sorting index in dairy cows. Eight rumen-cannulated Holstein cows (4 primiparous and 4 multiparous, 151.2 ± 61.4 DIM and 33.7 ± 3.0 kg/d milk yield) were blocked by parity, and enrolled in a 4×4 Latin Square (LS) design experiment. Each 21-d period included 7 d for sampling. Dietary uNDF was exclusively manipulated using corn silage (CS) from plants harvested at 2 heights (40 cm [CS40] and 65 cm [CS65]) and offered as TMR with a 48:52 forage-to-concentrate ratio. Treatments with decreasing uNDF (11.3%, 10.4%, 9.6%, and 8.9% DM) consisted of 0%, 33.3%, 66.6%, and 100% CS65, with the remainder as CS40. Feeding behavior was monitored over 48 h at 5-min intervals, tracking eating, drinking, lying, standing, and milking. Rumination was recorded via accelerometers. Orts and TMR were collected for particle size estimates. Data were analyzed by Mixed procedure of SAS modeling the fixed effects of treatment, square, and their interaction. Cow within square was considered as random effect. Treatment differences were analyzed by linear and quadratic contrasts. The reduction in dietary uNDF concentrations quadratically affected lying and ruminating times ($P \leq 0.034$). Standing time exhibited a negative linear tendency ($P = 0.077$). No significant effects were observed for eating, drinking, idling or chewing times. Time spent eating per kilogram of NDF and DM showed a negative quadratic effect, while it increased per kilogram of uNDF intake. Time spent ruminating and chewing decreased linearly per kilogram of NDF and DM but increased per kilogram of uNDF ($P < 0.001$). A negative quadratic effect was observed for the rejection of long feed particles ($P = 0.015$) as uNDF decreased. No selection for particles between 19 and 8 mm. A positive quadratic tendency was observed for selecting particles between 8 and 4 mm and those smaller than 4 mm ($P \leq 0.075$) for lower uNDF diets. Although dietary characteristics may affect feeding behavior, some cows have a preference for particle size.

Key Words: carbohydrate, fiber indigestible, rumination

2472 Evaluating feed sorting behavior and TMR composition in roughage intake control feeding systems. S. E. Green, U. Arshad, S. J. Kendall, K. M. Kennedy, E. C. Diepersloot, L. F. Ferraretto, and H. M. White^{*}, University of Wisconsin–Madison, Madison, WI.

The objective of this study was to assess feed sorting behavior in roughage intake control (RIC) feeding bins and the subsequent nutrient composition of the TMR across the feed. Lactating multiparous Holstein cows ($n = 64$ /replicate) were housed in a freestall facility with access to 32 RIC bins during 2 experimental phases (replicate 1 = 122 ± 23 ; replicate 2 = 78 ± 24 DIM $\pm$ SD). Feed samples were collected in 3-h staggered intervals across 3 feed days, in addition to TMR samples at 0 h and refusals at 24 h. Feed samples were analyzed using the WI Oscillating Particle Separator to determine particle distribution by oscillating a 117mm horizontal circumference at a frequency of 2.4 ± 0.08 Hz for 45 s. Feed was separated into 5 sieves (26.9, 18, 8.98, 5.61, 1.65 mm) and 1 collection pan. Particle composition at each time point was measured using the AF weight by sieve. The 29.6-mm sieve was excluded from data analysis due to confounding factors. Feed sorting behavior was characterized as deviation in TMR particle size composition from initial feeding. Each sieve time point was dried for 48 h at 60°C for DM analysis and subsequently ground through a 4-mm screen. Nutrient analysis on composite samples was performed with TMR NIR chemical analysis. Data for each replicate were analyzed separately (PROC MIXED, SAS, 9.4) for feed sorting (fixed effect of feed h, sieve, sieve $\times$ feed h, and random effect of bin; h as the repeated statement) and nutrient analysis (fixed effect of feed day quarter, sieve, sieve $\times$ feed day quarter, and random effect of bin; 0 h relative to feeding as the covariate). Proportion of feed at each particle size (sieve $\times$ feed h) differed ($P < 0.0001$) only within the last 12 h of the feed day for both replicates, during which cows had less ($P < 0.0001$) AF intake. Nutrient analysis for CP, undigested NDF, ethanol and water-soluble carbohydrates, starch, and ether extract did not differ ($P \geq 0.13$) across the feed day. Although particle size differed by the end of the feeding day, the lack of impact on nutrient composition indicates that use of RIC feeding bins did not introduce additional variance in nutrient consumption across the feed day.

Key Words: RIC bin, feed efficiency, feed intake

2473 Effects of dietary content of undegradable neutral detergent fiber (uNDF) and crude protein (CP) on lactation performance, rumen fermentation, and total tract digestibility (TTD) of Holstein cows. T. N. Turney^{*1}, J. Ono², R. J. Grant¹, S. Y. Morrison¹, and H. M. Dann¹, ¹William H. Miner Agricultural Research Institute, Chazy, NY, ²ZEN-NOH National Federation of Agricultural Cooperative Associations, Tokyo, Japan.

Holstein cows ($n = 16$, parity ≥ 2) were used to determine the effect of dietary uNDF at 240 h and CP content on lactation performance, rumen fermentation, and TTD. A 4×4 Latin square design (28-d periods) with a 2×2 factorial arrangement of treatments was used. Treatments were: (1) low uNDF (7.9%), low CP (14.4%; LULP), (2) low uNDF (7.5%), high CP (16.6%; LUHP), (3) high uNDF (9.7%), low CP (15.3%; HULP), and (4) high uNDF (9.5%), high CP (17.3%; HUHP). Dietary uNDF and CP differed by changing the forage-to-concentrate ratio or protein source in concentrates. Cows were housed in tiestalls, individually fed TMR 1 $\times$ /d, and milked 3 $\times$ /d. All cows were used to determine lactation performance on d 19 to 28 and TTD on d 22 to 25. Ruminally cannulated cows ($n = 8$) were used to characterize rumen fermentation on d 23 to 26. Data were summarized by period and analyzed by ANOVA with the MIXED procedure of SAS (version 9.4). The model contained fixed (uNDF, CP, time [when appropriate], interactions [uNDF, CP, time], square, period[square]) and random (cow[square]) effects. In general, uNDF and CP affected lactation performance and ruminal fermentation independently; there were no significant interactions. Additional CP in a high uNDF diet increased TTD of DM (interaction $P = 0.04$). Low

Table 1 (Abstr. 2473).

Item	LULP	LUHP	HULP	HUHP	SE	uNDF	CP	uNDF × CP
DMI, kg/d	27.4	28.6	27.5	27.9	0.6	0.34	0.04	0.26
DMI, % of BW/d	4.00	4.14	3.96	3.97	0.12	0.05	0.15	0.22
ECM, kg/d	45.1	46.5	43.6	43.4	0.8	<0.01	0.36	0.17
Fat, %	3.73	3.73	3.89	3.88	0.08	<0.01	0.93	0.96
True protein, %	2.94	3.00	2.99	3.02	0.04	0.08	0.02	0.43
ECM/DMI, kg/kg	1.65	1.63	1.59	1.57	0.03	<0.01	0.18	0.93
MNE, %	34.5	30.0	31.0	27.2	0.6	<0.01	<0.01	0.32
pH	6.3	6.2	6.3	6.2	0.1	0.60	0.06	0.82
VFA, mM	134	136	130	136	3	0.24	0.04	0.33
Ammonia N, mg/dL	1.6	4.2	2.9	6.7	0.5	<0.01	<0.01	0.18
TTD DM, %	72.6	73.8	70.4	73.2	0.4	<0.01	<0.01	0.04
TTD NDF, %	51.7	53.1	45.7	48.5	1.1	<0.01	0.05	0.48

uNDF diets improved DM intake (DMI), energy corrected-milk (ECM), feed efficiency, milk N efficiency (MNE), and TTD whereas lower CP diets improved MNE and decreased TTD. Dietary uNDF and CP can be considered separately when formulating rations within the ranges studied. Results of the experiment are shown in Table 1.

Key Words: uNDF, protein, degradability

2474 Effect of supplemental chromium propionate on milk yield of multiparous Holstein cows fed precalving through late lactation. R. H. Brown^{*1}, S. R. Tsang¹, and W. P. Weiss², ¹*Chemlock Nutrition, Cincinnati, OH*, ²*The Ohio State University, Wooster, OH*.

Chromium propionate (CrP) has been demonstrated to improve milk production in dairy cows. However, a vast majority of those trials have evaluated dairy cows only during the transition or early lactation periods. Our objective was to determine the impact of CrP (Nutrilock Chromium, Chemlock Nutrition) on milk yield when fed to multiparous Holstein dairy cows starting in the close-up period and continuing through late lactation. The study was conducted on a commercial dairy located in western Wisconsin from April 2023 to June 2024. The dairy was equipped with 8 voluntary milking system (VMS) robots (DeLaval, Sweden), which allowed control (CTL) and CrP supplemented cows to be comingled and fed a common diet except for pellets which either contained no additional supplement or contained CrP. The CrP was formulated to feed 1 mg per 0.45 kg of pellet. The VMS varied feed rate of pellet from 2.7 kg to 5.4 kg daily based on milk yield. The cows fed CrP (n = 85) were selected based on having an even ear tag number and CTL cows (n = 76) were selected based on having an odd ear tag number. Cows began on either the CrP or CTL pellet approximately 21 d before calving and continued through late lactation. Individual cows' 7-d average milk production was recorded weekly and milk yield was analyzed at 30-d intervals from 1 to 269 DIM with the 'lmer' function of the 'lme4' package in R using a model that included the fixed effect of treatment (CTRL or CrP), the random effect of cow, and DIM as a covariate. Group 1 indicated DIM 1–14, group 30 indicated DIM 15–44, group 60 indicated DIM 45–74, and so on. Milk yield was higher ($P < 0.05$) in the CrP-fed cows in group 90 (56.8 vs. 54.1 ± 0.98 kg/d), group 120 (54.4 vs. 50.6 ± 1.10 kg/d), and group 150 (51.9 vs. 48.7 ± 1.12 kg/d). In addition, milk production tended to be higher ($P = 0.06$) for the CrP group at group 1 (34.6 vs. 32.5 ± 0.79 kg/d, $P = 0.06$). No other points were significantly affected by CrP supplementation. This study shows the impact of CrP on milk production past the transition phase.

Key Words: chromium propionate, milk yield, late-lactation

2475 Factors influencing isoflavone content and optimization of extraction conditions in red clover. Z. B. Xiong, J. Q. Wang, and S. G. Zhao^{*}, *Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China*.

Red clover (*Trifolium pratense* L.) is a valuable leguminous forage, rich in bioactive isoflavones like biochanin A. This compound enhances rumen bacterial degradation, improves lactate metabolism, and helps mitigate subacute ruminal acidosis in ruminants. It boosts milk yield, composition, and feed efficiency in dairy goats and cows, while also positively influencing hormonal regulation, anti-inflammatory responses, and immune function in lactating cows. The purpose of this experiment was to explore the influencing factors of isoflavone content in red clover and the best extraction conditions by hot reflux. By selecting 4 different varieties of red clover (Reder, Xuanli, Agil, and Suprt), the isoflavones in leaves and stems were determined respectively to determine the influence of varieties and parts on the isoflavone content. Box-Behnken design was used to design a response surface experiment with 3 factors and 3 levels. The ratio of solid to liquid (1:6, 1:12, and 1:18 g/mL), extraction temperature (50°C, 75°C, and 100°C) and extraction time (0.5, 1.25, and 2 h) were the factors affecting the extraction efficiency, the yield of isoflavones from red clover was taken as the investigation basis, and 80% ethanol was used as the extraction solvent to determine the best extraction conditions. The quantification of isoflavones in red clover was performed using HPLC. The data were initially analyzed in Excel, then processed using Box-Behnken design and statistical analysis in Design Expert 13.0, with graphical visualization. The results showed that: (1) Xuanli was the highest variety, which was 5.92 g/kg DM, significantly higher than the total isoflavones of the other 3 varieties. The isoflavone content and mass ratio in leaves of red clover were significantly higher than those in stems. (2) The optimum extraction conditions were as follows: the ratio of solid to liquid was 1:18 g/mL, the extraction temperature was 90°C, and the extraction time was 1 h. The isoflavone yield was 93.71%. It can be seen that the variety of red clover significantly affects the isoflavone content, and the isoflavone enrichment degree in leaves is higher than that in stems.

Key Words: red clover isoflavone, extraction optimization, response surface analysis

2476 Purple-corn silage: I. Forage yield, nutrient composition, and concentration of anthocyanins from silage of conventional corn hybrid and its isogenic pigmented. O. I. Santana^{*1}, F. J. Santoyo Ávila², A. Peña Ramos¹, and J. I. Sánchez Duarte³, ¹*Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias*,

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The aim of this study was to evaluate yield, nutrient composition, and anthocyanins concentration at different days of fermentation of a conventional and its isogenic purple corn hybrid. A 4-replicated randomized complete design was used to assess a conventional white-dent kernel (WS) and its isogenic purple hybrid containing anthocyanins (PS; Supremo, Aspro). The study was done at the Agricultural Research Station of INIFAP (Pabellón, MX) located at 22.0934 N, 102.1733 W, and 1,905 masl. Each experimental unit was 4 rows at a distance of 0.76 m and 5.0-m length, where hybrids were grown at density of 87,200 plants/ha in a field naturally infested by *Fusarium* spp. When the kernel reached R5 stage (1,520 growing-degree units and 140 d), all plants in the 2 central rows were cut and thereafter weighed, then 5 whole-plants were randomly taken and chopped at 22 mm. A sample of 300 g was dried until constant weight at room temperature under darkness to determine DM and the initial concentration of anthocyanins. Mini-silos of 300 g each were made in bags using a vacuum to extract the air and sealing to ferment for 30, 60, and 90 d at room temperature. Silage dry samples were ground to analyze chemical composition, digestibility in situ at 48 h, and concentration of anthocyanins as cyanidin-3-glucoside. Yield of DM was 35 t/ha in WS and 33 t/ha in PS, but forage DM content did not differ between hybrids (40.9% ± 1.2% DM). Content of CP, NDF, starch, and ash was similar between hybrids averaging 8.1%, 40.1%, 30.3%, and 4.8% of DM, respectively. Digestibility was 80.3% and 80.0% of DM and 51.7% and 50.3% of NDF for PS and WS, respectively. Initial concentration of anthocyanins in PS was 5,900 mg/kg, which declined to 1,500, 800, and 780 mg/kg on d 30, 60, and 90 of fermentation, respectively. There were no plants affected by rot-stalk and both hybrids were highly tolerant to *Fusarium* spp. Overall, isogenic PS hybrid performed fairly similar to the WS hybrid, but the former had high concentration of anthocyanins that could have beneficial effects on dairy cows.

Key Words: anthocyanin, dairy cow, silage

2477 Effects of dietary flavonoids on production, metabolism, and inflammation in periparturient dairy cows. G. J. Combs^{*1}, T. A. Flemming¹, B. M. Goetz¹, E. J. Mayorga¹, B. M. Buol¹, S. E. Schuling², D. E. Schimek², E. A. Horst³, T. L. Harris³, A. W. Holloway³, and L. H. Baumgard¹, ¹Department of Animal Science, Iowa State University, Ames, IA, ²NutriQuest, Mason City, IA, ³Elanco Animal Health, Greenfield, IN.

Objectives were to evaluate the effects of *Scutellaria baicalensis* extract (SBE) on production, metabolism, and inflammation during dry-off (DO) and the ensuing lactation. Holstein cows (n = 63; parity 1.8 ± 1.1) were enrolled 12 d before DO (−64 d from calving) and assigned to control (CON; n = 31) or SBE (10 g/d; n = 32) diets from DO through 21 DIM, and productivity was monitored through 63 DIM. Effects of treatment, time, and treatment × time were evaluated using PROC MIXED in SAS. Although not affected by SBE, LPS-binding protein (LBP), serum amyloid A (SAA), and haptoglobin (Hp) increased 2 d post-DO (2.0-, 3.0-, and 13.5-fold, respectively; *P* < 0.01), and while LBP and SAA returned to pre-DO levels by 9 d DO, Hp remained elevated compared with pre-DO at 9 d (*P* < 0.01). After calving, LBP, SAA, and Hp peaked at 3 DIM (3.0-, 13.1-, and 23.3-fold from prepartum, respectively) and decreased as lactation progressed (*P* ≤ 0.01), with SBE supplementation slightly increasing Hp postpartum (65%; *P* = 0.02). Regardless of treatment, circulating glucose and insulin decreased at 7 DIM (16% and 67%, respectively), while NEFA and BHB increased 1.9- and 3.8-fold,

respectively. Fecal pH decreased 0.5 units after parturition similarly between treatments (*P* < 0.01). Milk yield (MY), DMI, and energy corrected MY increased after calving but were unaffected by SBE. Body weight (BW) and BCS decreased (*P* < 0.01) after calving, and SBE attenuated the post-calving BCS decline (*P* = 0.05) and tended to increase BW at 4–9 weeks post-calving (*P* = 0.09). Cows supplemented with SBE had an increased proportion of epithelial cells sloughed in milk (8%; *P* = 0.05) without SCC or SCS differences after calving (*P* > 0.35). From wk 4–9 of lactation, SBE-fed cows had increased milk protein content (3.4%; *P* = 0.05), decreased MUN (6.0%; *P* = 0.04), and increased circulating insulin (*P* = 0.01). Inflammation occurred following DO and calving, and although SBE did not influence inflammation, it did ameliorate BCS loss and increased BW.

Key Words: dry-off, immune activation

2478 Meta-analysis to determine the effects of supplementation of niacin during the transition or lactating period on performance in dairy cows. U. Arshad^{*1}, S. Bagaria¹, C. M. Ncho¹, M. Sagheer², K. C. Krogstad³, and M. Niu¹, ¹ETH Zurich, Zurich, Switzerland, ²University of Florida, Gainesville, FL, ³The Ohio State University, Wooster, OH.

Objectives were to determine the effects of feeding niacin during the transition or lactating periods on performance in dairy cows. A systematic review identified 41 experiments (128 treatment means, 1,992 cows) for meta-analysis. Each experiment included a nonsupplemented control (CON) group and a supplemented group, with niacin provided as rumen-unprotected (RUPN) or rumen-protected niacin (RPN). Data were categorized into 4 cohorts: RUPN or RPN supplementation during either the transition or lactating periods. Cows supplemented with RUPN during the transition (CON: 13 vs. RUPN: 14 treatment means) and lactating periods (CON: 33 vs. RUPN: 40 treatment means) consumed 13.9 ± 10.8 g/d and 9.1 ± 3.5 g/d niacin, respectively. For RPN, niacin intake during the transition (CON: 4 vs. RPN: 4 treatment means) and lactating periods (CON: 8 vs. RPN: 12 treatment means) was 11.7 ± 7.3 g/d and 4.6 ± 2.0 g/d, respectively. Mixed models assessed linear and quadratic effects, and meta-analytical statistics in STATA estimated weighted mean differences (WMD) for treatment comparisons, accounting for random experiment effects and weighting by the inverse of SEM squared. Niacin supplementation during the transition period did not enhance performance. However, RUPN supplementation during the lactating period linearly increased DMI and yields of milk, ECM, protein, and lactose and increments of 0.50, 0.90, 0.70, 0.04, and 0.05 kg/d, respectively, were observed with 9.1 g/d RUPN. Across 38 treatment comparisons, RUPN cows produced 0.83 kg/d more milk than CON cows (WMD = 0.83; 95% CI = 0.35–1.31) with moderate heterogeneity (*I*² = 61.7%). Similarly, RPN supplementation during the lactating period linearly increased DMI and yields of milk, ECM, fat, and lactose and increments of 0.60, 1.10, 0.70, 0.06, and 0.05 kg/d, respectively, were observed with 4.7 g/d RPN. Across 12 treatment comparisons, RPN cows produced 0.96 kg/d more milk than CON cows (WMD = 0.96; 95% CI = 0.62–1.31) with no heterogeneity. Overall, feeding 9.1 g/d RUPN or 4.7 g/d RPN during lactation improved milk production without improving feed efficiency.

Key Words: dairy cow, niacin, performance

2479 Antioxidant and mineral status of Jersey cows fed a diet with 3% inclusion of *Chondrus crispus* during the grazing season. W. M. Sims^{*}, M. A. Rahman, K. V. Almeida, M. A. Arshad, M. M. H.

Khandakar, M. G. M. Carvalho, O. Osuolale, M. L. Fischer, L. C. R. Junior, and A. F. Brito, *University of New Hampshire, Durham, NH*.

Seaweeds contain bioactive compounds and minerals that may improve the antioxidant and mineral status of dairy cows. *Chondrus crispus* (CC), a red macroalgae harvested in the northeastern United States, can be used to supply minerals for dairy cows. Further, carrageenan, the primary bioactive compound in CC, has been shown to enhance antioxidant status of nonruminant animals. We aimed to assess plasma concentrations of antioxidant enzymes and milk minerals using 12 multiparous and 8 primiparous organic Jersey cows (136 ± 32 DIM; 442 ± 52 kg BW) fed CC during the grazing season in a randomized complete block design. Cows were given either pasture and a partial total mixed ration (pTMR) with no seaweed (control = CON) or pasture and a pTMR with 3% DM inclusion of CC (seaweed = SWD). Diets were formulated to yield comparable levels of minerals. Cows had access to a grass-legume pasture mix (55.2% NDFom, 16.3% CP; herbage allowance 17.5 kg/cow daily) for 16 h and were given the pTMR (16% CP, 25% NDFom) indoors after a.m. and p.m. milkings. The pTMR contained 37% grass-legume mixed baleage and 63% of ground corn/soybean meal-based concentrate. The experiment lasted 10 wk, including 2 wk for covariate measurements followed by 2 sampling periods during wk 4 and wk 8. Data were analyzed in R with treatment, week, and interactions entered as fixed effects, and block as random effect. The emmeans function in R was used for pairwise comparison with significance set at $P \leq 0.05$ or tendency at $0.05 < P \leq 0.10$. Plasma concentration of superoxide dismutase ($P = 0.60$) and catalase ($P = 0.88$) did not differ between diets. Contrarily, cows fed CON tended ($P = 0.06$) to have greater plasma concentration of glutathione peroxidase than SWD (37.9 vs. 30.0 ng/mL). Milk concentration of Ca, P, Na, K, Cl, S, Mg, and I were similar ($P \geq 0.11$) across diets. However, SWD cows had greater milk Se concentration ($P < 0.01$; 65.3 vs. 61.5 ug/L), and tended to have greater milk Zn content ($P = 0.07$; 4.96 vs. 4.71 ug/L) than CON. Our data showed that SWD increased milk Se concentration, but did not improve the antioxidant status of grazing dairy cows.

Key Words: antioxidant, mineral, seaweed

2518 Feeding *Chondrus crispus*: Effects on ruminal fermentation, purine derivative excretion, and enteric methane emissions in grazing dairy cows. M. A. Rahman*, W. M. Sims, M. L. Fischer, M. A. Arshad, M. M. H. Khandakar, M. G. M. Carvalho, L. C. R. Junior, O. Osuolale, K. V. Almeida, and A. F. Brito, *Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, NH*.

We aimed to investigate the effects of the red seaweed *Chondrus crispus* on ruminal fermentation, urinary purine derivatives excretion, and enteric CH₄ emissions in dairy cows. Twelve multiparous and 8 primiparous Jersey cows (136 ± 32 DIM, 442 ± 52 BW) were used in a randomized complete block design. Cows had access to a grass-legume pasture mix (herbage allowance = 17.5 kg/cow daily) for 16 h/d and were housed for 8 h/d when they received a partial total-mixed ration (pTMR) and were milked. The pTMR was formulated to contain the following (DM basis): 37% mixed-mostly legume baleage and 63% of a ground-corn/soybean meal-based concentrate. Cows were paired ($n = 10$ pairs) based on DIM and parity and, within block, randomly assigned to 1 of 2 diets: (1) pasture plus pTMR (control = CON) or (2) pasture, pTMR, and 3% *C. crispus* (seaweed = SWD). Diets were formulated to be isonitrogenous and comparable in terms of mineral profile with a 60:40 forage:concentrate ratio. Pasture averaged 16.3% CP and 55.2% NDFom, with the pTMR averaging 16% CP and 25% NDFom. The experiment lasted 10 wk, with 2 wk for covariate measurements followed

by 2 sampling periods during wk 4 and wk 8. Two GreenFeed units were used to measure gaseous emissions. Spot urine samples were collected 10 times over 6 d, and ruminal fluid samples were taken via stomach tubing once daily (~0930 h, after the morning feeding). Diets did not ($P = 0.61$) affect the urinary excretion of total purine derivatives. Similarly, ruminal total VFA concentration did not differ ($P = 0.72$) between treatments. However, the ruminal molar proportions of acetate and butyrate decreased ($P < 0.01$), and that of propionate increased ($P = 0.04$) with feeding SWD versus CON. In addition, the ruminal acetate:propionate ratio decreased ($P < 0.01$) in cows fed SWD. Diets had no effect on CO₂, O₂, and H₂ fluxes. Contrarily, CH₄ production, CH₄ yield, and CH₄ intensity decreased ($P \leq 0.01$) by 9.6%, 14.5%, and 6.72% with feeding SWD versus CON. Overall, feeding 3% *C. crispus* altered ruminal fermentation and decreased enteric CH₄ emissions in dairy cows.

Key Words: methane, seaweed, pasture

2520 Incorporating *Hermetia illucens* larvae in Jersey cow diets: Effects on milk production and composition. E. O. Reyes, M. D. Osorio, J. Orozco, K. L. David, and M. Moncada-Láinez*, *Zamorano University, Zamorano, Honduras*.

The rising cost of traditional dairy cow feed ingredients, such as corn and soy, has driven the search for more sustainable and cost-effective alternatives. *Hermetia illucens* larvae, commonly known as black soldier fly larvae, have gained attention as a viable protein and energy source, with the added benefit of organic waste biodegradation. This study evaluated the effects of incorporating 10% and 15% *Hermetia illucens* larvae into the diets of Jersey cows on milk production and composition. The research was conducted at the Dairy Cattle Unit of Zamorano University, Honduras, over 2 mo, with an initial 2-wk adaptation period. Thirty Jersey cows in their second to fourth lactation were assigned to a completely randomized design (CRD) with 3 groups: a control group fed a conventional TMR and 2 experimental groups receiving TMR supplemented with different levels of *Hermetia illucens* larvae. The cows were managed in a semiconfined system, receiving TMR twice daily (0500 and 1500 h) for 3 h per feeding before being allowed to graze on Star-grass (*Cynodon nlemfuensis*) for the remainder of the day in an intensive rotational grazing system. Four key variables were analyzed: feed intake, milk production, body condition, and milk composition. Data were assessed using least squares means (LSMEANS) separation tests, with probability differences (PDIF) calculated via the Statistical Analysis System (SAS) version 9.4, applying a significance threshold of $P \leq 0.05$. Results show that a 15% inclusion of *Hermetia illucens* larvae increased milk yield by 1 kg compared with the control group ($P < 0.0001$), while the 10% group showed values similar to the control. However, the 15% inclusion reduced feed intake ($P = 0.0004$) and negatively affected body condition ($P = 0.0078$). In terms of milk composition, the group with the highest larvae inclusion exhibited a decrease in fat content ($P = 0.018$), while protein and nonfat solids remained comparable to the control group. These findings suggest that *Hermetia illucens* larvae could serve as a viable alternative feed ingredient for dairy cattle, offering potential cost savings without significantly compromising milk quality.

Key Words: alternative feeding, black soldier fly, milk yield

2521 Protein digestive kinetics of the newly developed value-added blended fat stimulated feed product in lactating dairy cows in comparison with commercial protein and energy feeds. U. Ihsan, M. Rodriguez Espinosa, L. L. Prate, and P. Yu*, *Department of Animal*

and Poultry Science, College of Agricultural and Bioresources, University of Saskatchewan, Saskatoon, Canada.

The objectives of this study were to compare commercial protein and energy products with the newly developed value-added blended fat stimulated feed product (BFSFP) on rumen degradation kinetics of protein in lactating dairy cows. Three batches of samples of the newly developed value-added blended fat stimulated feed products BFSFP1, BFSFP2, and BFSFP3 were developed. The only difference among the 3 BFSFP tested was batch. Three samples of barley grain ($n = 3$) and canola meal ($n = 3$) were obtained from Canadian Feed Research Center (CFRC, University of Saskatchewan, Canada). The rumen degradation kinetics of protein in fistulated lactating dairy cows were determined using the in situ technique. The treatment design was a one-way structure with 3 feed treatment levels ($i = 1$ [BFSFP], 2 [barley], 3 [canola meal]). The experimental design was a randomized complete block design (RCBD) with the feed treatments as a fixed effect ($i = 1$ [BFSFP, $n = 3$], 2 [barley, $n = 3$], 3 [canola meal, $n = 3$]) and in situ experimental runs as a random block effect. The results showed that compared with commercially available energy-rich and protein-rich feeds, the newly developed BFSFP was lower in the degradation rate of crude protein (K_d) than barley grain (3.76% vs. 10.42%/h, $P < 0.05$), but not significantly different from canola meal ($K_d = 7.27\%/h$). The BFSFP was higher ($P < 0.05$) in potentially degradable fraction (D) than barley grain and no difference from canola meal (D: 38.6% vs. 26.4% vs. 31.3%, respectively). The BFSFP had higher in rumen bypass protein (BCP: 87 vs. 21 and 73 g/kg DM, respectively) and effective rumen-degradable protein (EDCP: 231 vs. 106 vs. 350 g/kg DM, respectively) when compared with barley grain and canola meal. In conclusion, the BFSFP has different protein digestive kinetics compared with the commercially available energy-rich and protein-rich feeds.

Key Words: blended fat stimulated feed product, digestive kinetics, lactating dairy cow

2522 Effect of adding plant extracted hydrolyzable tannin and saponin combination on total digestible nutrient, energy values, and feed milk values of blend pelleted products for dairy cows. T. Hidayat, M. Rodriguez Espinosa, and P. Yu*, *Department of Animal and Poultry Science, College of Agricultural and Bioresources, University of Saskatchewan, Saskatoon, Canada.*

The objectives of this study were to determine effect of adding plant extracted hydrolyzable tannin (HT) and saponin (S) combination as a combined phytochemical feed additive (HTS) on total digestible nutrient, energy values, and feed milk value (FMV_{NEL}) of blend pelleted products for dairy cows. The plant extracted HT and S were combined with 4 different levels, which included level 1 = 0 (control, 0%HT+0%S of diet DM), level 2 = 1.5 (1%HT+0.5%S of diet DM), level 3 = 3 (2%HT+1%S of diet DM), and level 4 = 4.5 (3%HT+1.5%S of diet DM). Then these combined additives (HTS) were added to canola meal and pea mixtures with 2 different ratios of canola meal to pea: 50:50 and 70:30. Total 8 blend pelleted feed products (= 4 additive levels $\times$ 2 ratios of canola meal and pea mixtures = 8) were produced at Canadian Feed Research Center (CFRC, University of Saskatchewan, Canada). The total digestible nutrients (TDN) and energy values (ME, NE) were evaluated using NRC chemical composition to estimate. Based on chemical composition of the products, the values of TDN, ME, and NE were calculated. The FMV_{NEL} was determined based on NEL. The experimental design was a completely random design (CRD) with the additive levels and 2 ratio of blend pellet products as fixed effects. The data were analyzed using the Mixed Model procedure of SAS. Orthogonal polynomial contrast in SAS was used to determine linear and quadratic relationship between

dosage level and nutritional values (TDN, ME, NE, FMV_{NEL}). The results showed that adding HTS had a quadratic effect on total digestible nonfiber carbohydrate (tdNFC with means from 42% to 44% DM, $P = 0.037$) and total digestible crude protein (tdCP with mean from 32% to 34% DM, $P = 0.060$), TDN (with mean from 74% to 76% DM, $P = 0.062$), ME (mean from 2.90 to 2.95 Mcal/kg, $P = 0.063$), and FMV_{NEL} (with mean from 2.65 to 2.71 kg milk/kg product, $P = 0.063$). Compared with 2 different ratios of blend pellet products, it showed that the blend pellet product with 50:50 ratio of canola meal to pea had higher ($P < 0.01$) TDN (76.8% vs 73.8%DM), NEL (1.89 vs. 1.83 Mcal/kg), and FMV_{NEL} (2.70 vs. 2.62 kg milk/kg feed). In conclusion, adding plant extracted HT and S combination to canola meal and pea mixtures affected TDN, ME, NE, and feed milk value.

Key Words: phytochemical feed additive, energy value, feed milk value

2523 Green economy: Incorporating spent mushroom substrate into dairy cattle diets for environmental sustainability. J. O. Alabi*, O. O. Adelusi, M. Wuaku, C. C. Anotaenwere, D. O. Okedoyin, O. A. Oderinwale, J. M. Enikuomehin, A. E. Kholif, and U. Y. Anele, *North Carolina A&T State University, Greensboro, NC.*

Spent mushroom substrate (SMS) presents a cost-effective and nutrient-rich alternative feed resource for cattle production. This study examined 3 types of spent *Pleurotus* mushroom substrates (SMS1, *P. ostreatus* isolate 1; SMS2, *P. pulmonarius*; and SMS3, *P. ostreatus* isolate 3) using in vitro fermentation. The experiment used a $3 \times 3 + 1$ factorial design to assess 3 inclusion levels (10%, 20%, and 30%) as substitutes for corn silage. Four rumen-cannulated nonlactating dairy cows were used as inoculum donors. The results revealed a significant interaction ($P < 0.001$) between SMS type and inclusion level on nutrient digestibility. Specifically, SMS3 at 30% inclusion level increased neutral detergent fiber digestibility by 14% and 7%, while ADF digestibility increased by 20% and 18% at 24 and 48 h, respectively, compared with corn silage. At 48 h, ADL digestibility was 106% higher in the SMS3 group at 30% compared with the control. Additionally, fiber digestibility increased linearly ($P < 0.001$) with higher SMS inclusion levels. Main and interaction effects of SMS type and inclusion level had no significant effect on GHG and VFA production at 24 and 48 h. The pH was positively influenced ($P < 0.01$) by SMS inclusion at 48 h only, with higher pH observed in SMS 3 at 10% level. However, SMS inclusion reduced ($P < 0.001$) gas production from 24 to 48 h. In conclusion, incorporating SMS into dairy cattle diets enhances nutrient digestibility without negatively affecting VFA production, making SMS a viable feed resource for sustainable dairy production.

Key Words: spent mushroom substrate, *Pleurotus* spp., sustainability

2524 In vitro and in situ evaluations of black soldier fly larvae as a methane-mitigating protein substitute in dairy cattle diets. H. Craig*¹, A. Tiwari², R. Kohn², J. E. Rico^{3,2}, and B. Lamp¹, ¹Department of Entomology, University of Maryland, College Park, MD, ²Department of Animal and Avian Sciences, University of Maryland, College Park, MD, ³Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA.

Black soldier fly larvae (*Hermetia illucens*; BSFL) meal is a high-quality protein product, compositionally on par with soybean meal (SBM). Previous in vitro work suggests BSFL may mitigate enteric CH₄. Different BSFL products are available with fractions that vary in the concentration of nutrients (i.e., fat and protein) and antinutritional factors (e.g., chitin). Our objectives were to (1) determine the optimal

inclusion rate of low- or high-fat BSFL meal to mitigate CH₄ in vitro (experiment 1; EXP1), and (2) compare the degradation rates of BSFL and SBM in vivo (EXP2). For EXP1, a batch culture system was used in a dose-response study testing low- (11.6% crude fat; LF) or high-fat (34.9% crude fat; HF) BSFL at 4 inclusion levels (0%, 10%, 25%, and 50% of DM). The experimental treatments were allocated in a randomized design with a 2 × 3 factorial structure. Each treatment was replicated 3 times across 3 independent experiments. Volatile fatty acids and CH₄ were measured using GC. For EXP2, nylon bags with 5.0 g of either BSFL or SBM were incubated in the rumen of 2 fistulated Holstein cows and DM disappearance was evaluated at 0, 2, 4, 6, 8, 10, 12, 16, 20, 24, and 48 h of incubation. Data for EXP1 and EXP2 were analyzed using mixed models with fixed and random effects, and their interactions. In

EXP1, CH₄ increased over time ($P < 0.05$), and no treatment differences were detected for dose or fat type ($P = 0.98$ and $P = 0.76$, respectively); however, propionate and butyrate concentrations were higher in LF ($P < 0.05$). In EXP 2, DM cumulative degradation was higher in SBM relative to BSFL ($P < 0.05$), and DM disappearance rates were faster for BSFL from h 0 to 10, and slower between h 20 to 48, relative to SBM ($P < 0.05$). Although our findings do not support BSFL as an effective methane mitigating feed, our fermentation data indicates that it may still provide nutritional value. Further research is needed to determine the optimal use of BSFL in sustainable dairy diets.

Key Words: black soldier fly larvae, methane, sustainability

Ruminant Nutrition 2: Proteins and Amino Acids—Posters

2480 How does extra rumen-protected protein affect the influence of nitrate and tannin supplementation on nitrogen utilization and plasma metabolites? R. Peng^{*1}, S. E. Räisänen², R. Rauch³, A. Rytz³, E. Soussan³, M. Bobkov³, M. Kreuzer¹, and M. Niu¹, ¹*Institute of Agricultural Sciences, Department of Environmental Systems Science, ETH Zürich, Zürich, Switzerland*, ²*Department of Animal and Veterinary Sciences, Aarhus University, Tjele, Denmark*, ³*Nestlé Institute of Agricultural Sciences, Nestlé Research, Lausanne, Switzerland*.

We examined the effects of nitrate and *Acacia mearnsii* tannins (AMT), alone, combined, and with extra protein (rumen-protected rapeseed meal, RPR) on nitrogen utilization efficiency (NUE) and plasma metabolites, in cows fed a 14%-CP diet (on DM basis). We also assessed whether nitrate use results in nitrate and nitrite residues in milk or changes in hematological parameters. Eighteen Holsteins (203 ± 60.5 DIM, 33 ± 5.0 kg/d milk yield) were subjected to a split-plot design with nitrate (0.62% of DM) or iso-nitrogenous urea as main plot treatments. Each cow followed a replicated 3 × 3 Latin Square with 3 21-d treatments: (1) CON (basal diet), (2) AMT (3% of DM), and (3) AMT+RPR (6.4% of DM). Feed, milk, urine, feces, and blood were sampled at the end of each period. Data were analyzed using a linear mixed model. Milk nitrate and nitrite concentrations remained unaffected by treatments (overall 3.4 ± 0.97 and 0.04 ± 0.029 mg/L, respectively). Blood MetHb increased marginally with nitrate but remained within safety limits (peak: 3.06% of Hb). Interactions occurred between main plot and subplot treatments only for BUN and plasma nonesterified fatty acid (NEFA). Nitrate improved NUE by 11%, driven by an 11% greater (trend) milk N secretion at unchanged N intake. Conversely, AMT reduced NUE by 4%, and milk N secretion by 8%. Extra RPR with AMT further reduced NUE by 10%, as the extra N consumed was not utilized for milk protein synthesis. Urinary N excretion decreased by 28% and fecal N excretion increased by 44% with AMT. Extra RPR with AMT further elevated fecal N and tended to increase urinary N. With nitrate, AMT tended to reduce BUN and increase plasma NEFA, while extra RPR reversed this effect. Plasma Ala increased with AMT, while extra RPR reduced Gly, suggesting elevated urea cycle activity. Replacing urea with nitrate improved NUE without adversely affecting blood MetHb, while AMT shifted N excretion from urine to feces. Extra RPR decreased NUE and did not alleviate the negative effects of AMT on milk yield, suggesting that diets were not protein-deficient.

Key Words: nitrogen, milk quality

2481 Advancing recovery strategies for 2-hydroxy-4-methylthiobutanoic isopropyl esters (HMBi): Long-term stability of HMBi in pelleted concentrate feed. B. Vennekens, B. Janssens, E. E. Gültepe*, D. Martínez Del Olmo, F. Kiekens, and B. Forier, *Kemin Animal Nutrition and Health EMENA, Herentals, Belgium*.

Methionine analogs offer an alternative to encapsulated forms due to their stability during handling or thermo-physical processing. While HPLC UV has been used to assess the stability of isopropyl esters of hydroxy methionine analogs (HMBi) in pelleted feed, the need for a precise and efficient approach has emerged due to challenges in recovery from complex feed matrices and labor-intensive in/ex vivo methods. Building on our previous studies, this research aimed to develop an optimized extraction and analysis method via liquid chromatography-mass spectrometry (LC-MS/MS) to evaluate HMBi in pelleted concentrate

feed over 110 d. A high concentrated liquid HMBi product (≥97% HMBi, KESSENT MF (KESS), Kemin Animal Nutrition and Health, Belgium) was incorporated at 3 kg/ton into a grain- and soybean-based feed containing 18% crude protein before pelleting. The basal diet (without product) was also pelleted and served as a control. Concentrations of HMBi were analyzed on d 19 (short-term, n = 1) and 110 (long-term, n = 3) of storage at 16 ± 4°C. Liquid extraction was followed by LC-MS/MS analysis. Method capabilities were tested using replicate analyses and a mixed spike approach with final product and hydroxy methionine analog (2-hydroxy-4-methylthiobutanoic acid, HMBa) and HMBi standards. The method showed high linearity ($R^2 \geq 0.99$) for both neat solvent and pelleted feed extract with minimal matrix effect. Pre-extraction laboratory spike recoveries of the liquid HMBi product on the basal diet confirmed complete HMBi recovery from the pellet feed matrix (104.8% on d 19 and 107.6 ± 2.4% on d 110). As an innovative part of the LC-MS/MS based approach, concurrent HMBa and HMBi analyses quantified the hydrolysis of HMBi into HMBa under the storage conditions. During storage, the intact HMBi recovery decreased with 4.8%, while hydrolysis into HMBa remained minimal (0.5% increase). The developed LC-MS/MS method enabled full HMBi recovery from the pelleted feed matrix. Furthermore, it showed that hydrolysis of HMBi into HMBa in KESS was marginal during storage of pelleted feed that was treated with liquid HMBi product at 3 kg/ton. These findings confirm the stability of liquid HMBi in KESS in pelleted feed over long-term storage, demonstrating the effectiveness of this methodology.

Key Words: HMBi, pelleted feed, methionine

2482 Impact of rumen-protected methionine and genetic merit on milk protein and fat yield in dairy cows. J. P. A. Rezende^{*1}, G. M. de Souza¹, C. R. Monteiro¹, V. A. de Oliveira¹, J. H. Carneiro^{1,2}, and M. A. C. Danes¹, ¹*Universidade Federal de Lavras, Lavras, MG, Brazil*, ²*Dairy Inside, Castro, PR, Brazil*.

This study aimed to investigate the interaction between rumen protected methionine (RPM) supplementation and genetic merit for protein (PTAProt) or milk fat (PTAFat). Data from 3 experiments with RPM supplementation for at least 6 wk were used. The dataset included 337 cows with genomic information, 168 of which received RPM. Control received 65.2 g/d of metabolizable Met and 2.07% of Met as percent of metabolizable protein, while the RPM had 77.5 g/d and 2.46. Cows were grouped into 3 clusters based on PTAProt or PTAFat, ensuring $R^2 > 0.8$ between intracluster and total variance. For PTAProt (kg/lactation), cluster means were 1.28 (L, n = 67), 7.6 (M, n = 203) and 13.8 (H, n = 67). For PTAFat (kg/lactation), -0.37 (L, n = 67), 9.65 (M, n = 203) and 19.97 (H, n = 67). Variables $\Delta\%$ and Δ g/d were calculated as wk 6 value – covariate, while Δ g/d % of covariate was obtained by dividing Δ g/d by the g/d value of the covariate. Data were analyzed in PROC MIXED (SAS), with fixed effects of RPM supplementation, cluster (PTAProt for protein variables or PTAFat for fat) and their interaction, plus random effects of experiment and block nested within experiment. Covariates were included for protein and fat content and production, but not for Δ variables. Supplementation of RPM positively affected milk protein and fat content and yield (Table 1). Cluster effect was significant for milk fat (% or g/d), with higher PTA resulting in higher yield. No significant interaction between RPM and cluster was detected.

Key Words: genomic, methionine response

Table 1 (Abstr. 2482). Effect of RPM and cluster by PTAProt or PTAFat on milk protein and fat content and yield

Item	Control			RPM			SEM	P-value		
	L	M	H	L	M	H		RPM	Cluster	RPM × cluster
Milk protein										
%	3.25	3.24	3.27	3.35	3.36	3.41	0.04	<0.01	0.10	0.67
Δ%	0.06	0.05	0.07	0.16	0.18	0.21	0.03	<0.01	0.22	0.71
g/d	1,673	1,674	1,710	1,725	1,756	1,760	56	<0.01	0.32	0.59
Δg/d	−1	19	−8	44	58	72	44	<0.01	0.82	0.70
Δg/d, % of covariate	0.8	−0.6	−0.4	3.5	4.0	5.0	3.0	<0.01	0.87	0.66
Milk fat										
%	3.03	3.30	3.31	3.22	3.37	3.47	0.23	0.01	<0.01	0.54
Δ%	0.01	0.04	−0.03	0.00	0.15	0.25	0.09	0.01	0.22	0.09
g/d	1,520	1,693	1,748	1,643	1,712	1,794	127	<0.01	<0.01	0.43
Δg/d	−61	−20	−51	−23	42	96	53	<0.01	0.22	0.34
Δg/d, % of covariate	−2.2	−0.3	−0.9	0.1	2.9	6.3	3.1	0.01	0.29	0.50

2483 Effects of diversifying protein sources on the response of high-producing dairy cows to rumen-protected methionine. G. M. de Souza^{*1}, J. P. A. Rezende¹, V. A. Oliveira¹, M. C. S. Santos¹, L. C. B. Pimenta¹, J. H. Carneiro², F. Lopes³, and M. A. C. Danes¹, ¹*University of Lavras, Lavras, MG, Brazil*, ²*Dairy Inside, Castro, PR, Brazil*, ³*Adisseo USA Inc., Alpharetta, GA*.

Methionine (Met) is often the most limiting AA in lactating cow diets. However, the response to rumen-protected methionine (RPM) supplementation may depend on the supply of other AA. This study evaluated whether combining protein sources (PS) enhances dietary protein utilization and improves the response to RPM. Holstein cows (131 ± 82 DIM; n = 258) from a commercial herd were assigned to 4 treatments in a 6-wk randomized trial with a 2 × 2 factorial design. Factors were: (1) PS, soybean meal as the primary protein source (SOY) or a blend of soybean meal, canola meal, and distillers grains (BLEND); and (2) RPM (Smartamine M; 26–29 g/d) or control (CTL). Milk yield was recorded daily and composition analyzed at wk 3 and 6. Data were analyzed using a mixed model with PS, RPM, and their interaction as fixed effects and block and cow as random effects. There was no interaction between PS and RPM. Milk yield (58.0 kg/d) and lactose yield (2,756 g/d) were unaffected by treatments. However, energy-corrected milk was higher with RPM (CTL: 54.5 vs. RPM: 56.1 kg/d; *P* < 0.01). Milk fat yield was lower in BLEND than SOY (1,729 vs. 1,907 g/d; *P* < 0.01) but increased with RPM in both diets (CTL: 1,818 vs. RPM: 1,896 g/d; *P* < 0.01). Protein yield was lower in SOY-fed cows (1,882 vs. 1,957 g/d; *P* = 0.02) and increased with RPM (CTL: 1,920 vs. RPM: 1,988 g/d; *P* < 0.01). Casein yield followed a similar pattern, with lower values for SOY (1,480 vs. 1,539 g/d; *P* = 0.03) and an increase with RPM (CTL: 1,510 vs. RPM: 1,560 g/d; *P* < 0.01). Milk urea nitrogen was lower in BLEND than SOY (9.8 vs. 11.7 mg/dL) but increased with RPM (CTL: 10.8 vs. RPM: 12.1; *P* < 0.01). De novo fatty acids (FA; g/100 g milk) were lower in BLEND than SOY (0.54 vs. 0.67; *P* < 0.01) but increased with RPM (CTL: 0.61 vs. RPM: 0.65; *P* < 0.01). Mixed FA showed a similar response, while preformed FA was influenced only by PS (*P* < 0.01). Diversification of protein sources increased milk protein yield but reduced fat production, while the response to higher Met flux did not interact with diet and was consistently positive.

Key Words: amino acid nutrition, dietary protein optimization

2484 Effects of amino acid supplementation on the performance of lactating dairy cows: A meta-analysis. M. R. Villalobos

Barquero^{*1}, M. E. Van Amburgh¹, and K. F. Reed², ¹*Cornell University, Ithaca, NY*, ²*USDA-ARS Dairy Forage Research Center, Madison, WI*.

This study evaluates the impact of amino acid (AA) supplementation on the performance of lactating cows by identifying and quantifying variables influencing the effectiveness of lysine (Lys) and methionine (Met) supplementation. Fourteen studies were included in this analysis and categorized based on the supplemented AA: Met alone, Lys alone, or in combination (Met+Lys). A stepwise forward selection process was used to determine the best model fit. Studies were set as a random variable, while fixed variables included metabolizable energy balance (ME; energy required minus energy supplied, Mcal), metabolizable protein balance (MP; protein required minus protein supplied, g), and grams of AA supplied per Mcal of energy (g AA/Mcal). Production responses were measured as the mean differences between treatment and control groups. These included milk yield (MY, kg), energy-corrected milk (ECM, kg), and milk fat and protein (yield and percentage, kg and %). Our analysis showed that while MY and ECM improved with supplementation of both Lys and Met, Lys alone did not produce a significant response. Supplementing both Met and Lys together resulted in a MY increase of 3.7 kg per unit increase in AA delivery (g/Mcal), while ECM increased by 2.6 kg. Postpartum ME balance showed a slight negative association with differences in both milk yield and ECM for Lys and Met, suggesting that when energy is the primary limiting factor, AA supplementation does not enhance MY. Further exploration is needed, however, to understand the interaction between the stage of lactation and ME balance. Conversely, postpartum MP balance had a positive association with differences in MY and ECM for both Lys and Met, highlighting the role of AA supplementation to support greater MY and ECM when sufficient total MP is available. These findings emphasize the role of postpartum nutrient balance in shaping production responses to Lys and Met supplementation.

Key Words: amino acid, milk yield, nutrient balance

2485 Increased MP supply while maintaining dietary CP affects bacterial N recycled from BUN. T. Fernandes^{*1}, M. H. De Oliveira^{1,2}, J. D. De Oliveira^{1,3}, R. Hopkins¹, and M. D. Hanigan¹, ¹*Virginia Tech, Blacksburg, VA*, ²*State University of São Paulo, Botucatu, SP, Brazil*, ³*Federal University of Grande Dourados, Dourados, MS, Brazil*.

This research evaluated the effect of increasing MP supply by reducing RDP and increasing RUP while maintaining a constant CP level in the

diet on ruminal microorganisms N incorporation from N-urea recycling and amino acids absorption. Six ruminal cannulated Holstein heifers (398 ± 28 kg BW) were allocated to a replicated 3×3 Latin square design. Dietary treatments were balanced to provide: Low (9.85% MP), Medium (10.9% MP), and High (12.1% MP) MP levels, while maintaining iso-protein (16% CP) and isocaloric (2.62 ME, Mcal/kg) diets. Soybean meal and expeller were used as CP source. Each period was 14 d long with 10 d of adaptation followed by 4 d of jugular infusion of ^{15}N -urea double-labeled and with 12 h jugular infusion of U- ^{13}C -labeled AA mix and U- $^{13}\text{C}_6$ -labeled His on d 14. Rumen content and blood samples were collected during the infusions. Amino acids (Met, Thr, Val, Ala, Asx, Glx, Gly, Pro, and Ser) from rumen liquid-associated bacteria were assessed for atom percentage excess (ape) to represent the incorporation of ^{15}N recycled from BUN. Plasma AA absorption rates were derived for EAA (His, Ile, Leu, Lys, Met, and Phe) and NEAA (Ala, Gly, Pro, Ser, and Tyr) from plasma ^{13}C AA enrichment. Data were analyzed in R software, model had treatment and period as fixed effect, and cow nested in square as random effect. Treatment effects were evaluated by orthogonal polynomial contrasts. There was a linear increase ($P < 0.05$) in ape from low to high MP for Thr (3.26 to 5.08), Asx (4.09 to 6.30), Glx (4.15 to 6.45), and Ser (3.50 to 5.22). However, we observed a quadratic effect for Pro ($P = 0.02$); medium MP had a higher incorporation of N from BUN (2.84 ape in medium vs 2.05 Low and 1.22 in High MP). No effect was observed for Met, Val, Ala, and Gly. Only His plasma absorption rate was linearly affected by the diets, which were higher in the medium and high MP diets (114 g/d for both levels) vs. low MP (96 g/d). Increasing levels of MP by exchange RDP for RUP reduces the N available for ruminal bacteria and, consequently, increases liability on incorporation from N-urea recycled from BUN, with minor effects on plasma AA absorption.

Key Words: AA supply, isotope, rumen microorganism

2486 Comparative analysis of the impact of 2-hydroxy-4-(methylthio)butanoic Acid (HMTBa) and its salt variant on in vitro ruminal fermentation dynamics. M. Romero Huelva¹, I. Rivelli¹, D. R. Yáñez-Ruiz¹, E. Ramos Morales¹, D. Martínez Del Olmo^{*2}, L. De Grave², E. E. Gültepe², and B. Forier², ¹Estación Experimental del Zaidín, CSIC, Granada, Spain, ²Kemin Europa NV, Herentals, Belgium.

The study examines the effects of dry HMTBa on in vitro rumen fermentation, comparing it to its calcium salt counterpart using batch culture methodology. The HMTBa sources included KEMSSER H (KEM, 61.6% HMTBa, Kemin Animal Nutrition and Health, Belgium) and a commercial HMB-Ca salt (RS, $\geq 84.0\%$ HMTBa and its calcium salt). In vitro batch culture of 6 and 24 h were conducted with rumen fluid from cannulated sheep. The RS and KEM were incubated at 24 mg (providing 20.2 mg and 14.8 mg HMTBa, respectively) in 50 mL of diluted rumen fluid, reflecting their respective concentrations. Doses were formulated to align with typical dairy cow supplementation (24 g/day per head). The control group received an unsupplemented basal diet. Measured parameters included gas pressure kinetics, volatile fatty acids (VFA), lactic acid, $\text{NH}_3\text{-N}$, and pH. Data were analyzed using a randomized block ANOVA, with individual animals as blocking factors, and post hoc comparisons performed via Fischer's LSD test. Supplementation of KEM did not significantly change functional VFA and lactate productions, pH, N-NH_3 , and acetic:propionic acid ratio, and total gas production at both time points, while total gas pressure at 24 h was significantly higher ($P < 0.001$) for KEM compared with RS (10.1 vs. 9.64 ± 0.15). Isovaleric acid production was significantly lower in RS groups than in the control and KEM ($P = 0.017$), while no

treatment effect was observed for the rest of VFA. At 6 h, both lactate and N-NH_3 were lower in treatment groups compared with control (3.91 vs. 6.73 ± 0.55 ; 4.48 vs. 4.88 ± 0.14 , respectively) as a result of alteration in nitrogen and energy metabolism ($P < 0.033$), while no difference was observed among the groups after 24 h of incubation. In conclusion, the acid form of HMTBa supplementation shows similar effects to the Ca-salt form in the rumen even in case of lower delivery of equivalent HTMBa. The higher solubility of pure HTMBa, compared with the ion-bound Ca-salt form, may enhance microbial utilization in the rumen, allowing effective biohydrogenation and milk fat production at a lower delivery level. Further trials are needed to confirm this.

Key Words: analog, methionine, rumen fermentation

2487 Effects of rumen-protected methionine supplementation on the lower gut metabolome of dairy cows. W. M. Coelho Jr.^{*1}, M. J. Wollentarski¹, D. B. Melo¹, H. F. Monteiro¹, T. J. Almand¹, M. F. Vetter¹, M. F. Caballero¹, S. Olivarez¹, J. V. R. C. Alves¹, E. Neto², J. Hergenreder², J. Vasconcelos², F. Sun², and F. S. Lima¹, ¹Department of Population Health and Reproduction, University of California, Davis, Davis, CA, ²Kemin Industries, Inc., Des Moines, IA.

The lower gut plays a role in microbial nutrient utilization and metabolite production, essential for dairy cow health and performance. However, the effects of rumen-protected methionine (RPM) supplementation on the lower gut metabolome remain unexplored. This study evaluated RPM's impact on the lower gut metabolome of dairy cows. Thirty Holstein cows at 245 ± 3 d of gestation were blocked by parity (primiparous vs. multiparous) and randomly assigned within each block to no RPM (CON, $n = 15$) or RPM (KST, KESSENT Me, Kemin Industries Inc., $n = 15$). Cows were individually fed using Calan gates. The KST group received 5 g/d of RPM prepartum (27 ± 3 d) and 45 g/d postpartum (47 ± 3 d) to meet 100% of metabolizable methionine requirements, maintaining a 3:1 lysine-to-methionine ratio. Fecal samples were collected at 24 ± 3 d prepartum (baseline), wk 1 postpartum (W1), and wk 6 postpartum (W6) and analyzed using untargeted metabolomics at the West Coast Metabolomics Center via gas chromatography-time-of-flight mass spectrometry. MetaboAnalyst 6.0 was used for normalization and statistical analysis. A total of 420 metabolites were identified, including 158 known metabolites. No significant metabolite differences were observed at baseline. At W1, 21 metabolites were upregulated in KST compared with CON, with the highest variable importance in projection (VIP) scores observed for pipercolinic acid, tocopherol gamma, 1-monoolein, tocopherol α , cadaverine, glycolic acid, 3-hydroxyphenyl, heptadecanoic acid, maltotriose, 3,4-dihydroxybenzoic acid, glutaric acid, lactic acid, myo-inositol, and 2'-deoxyguanosine. At W6, 14 metabolites were upregulated in KST, with the highest VIP scores for ribonic acid, 1-kestose, 3,6-anhydro-D-galactose, 2'-deoxyguanosine, lactose, 5-aminovaleric acid, 2-deoxytetronic acid, N-acetyl-D-galactosamine, adipic acid, and urea. Pathway analysis indicated that galactose metabolism, lysine degradation, and glycerolipid metabolism were the most impacted by RPM. These findings suggest RPM supplementation influences the lower gut metabolome, modulating key metabolic pathways in dairy cows.

Key Words: metabolomics, methionine, fecal analysis

2488 Comparison of 2 methionine precursor sources in dairy goats using the area under the curve method. D. Martínez Del Olmo^{*1}, J. Mateos Aguado², E. Raffrenato², E. E. Gültepe¹, M. Hassan³, I. Rivelli³, D. R. Yáñez-Ruiz³, and M. Romero Huelva³,

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The kinetics and bioavailability of methionine analogs in dairy cows have been well documented in the literature. However, data on the utilization of the methionine precursor 2-hydroxy-4-(methylthio) butanoic isopropyl esters (HMBi) in dairy goats is scarce. This study evaluated the relative bioavailability of 2 HMBi products obtained through different production processes using the area under the curve (AUC) method in goats. The methionine precursors evaluated were the new HMBi product (KESSENT MF Dry, Kemin) and an existing HMBi product (Metasmart, Adisseo). Fifteen adult nonpregnant Murciano-Granadina goats, fed a 30:70 forage:concentrate diet, were assigned to a triplicate 3 × 3 Latin square design. Each period included 7 d: 2 experimental days and a 5-d washout. Three diets were evaluated: a negative control (NC), without methionine analogs, and 2 HMBi sources: KESSENT MF Dry (KMF) and Metasmart (MtS), both providing 6 g methionine equivalent. Treatments were dosed on d 1 of each period via esophageal tube directly into the rumen. Blood samples from the jugular vein were taken at various time points (0, 1, 2, 3, 4, 6, 9, 12, and 24 h) to measure plasma methionine concentrations. The elemental trapezoidal AUC was calculated by multiplying the mean methionine increase between consecutive samplings and the interval duration. The AUC was calculated for each treatment as the sum of the elemental AUC calculated all along the sampling kinetics. The AUC and plasma methionine concentrations were analyzed using an ANOVA. No significant differences ($P > 0.05$) for the total AUC were found between the KMF and MtS treatments (3,548 vs. 3,397 ± 251 arbitrary units). Plasma methionine concentrations reached their maximum value after 4 and 3 h of supplementation for KMF and MtS (274 ± 21.4 vs. 247 ± 18.9 μM), respectively. Both HMBi sources returned to a similar basal concentration (37.8 ± 9.5 μM) after 24 h of supplementation ($P = 0.422$). In conclusion, KESSENT MF Dry demonstrated similar bioavailability to Metasmart, in line with findings from previous studies conducted in dairy cows.

Key Words: bioavailability, methionine, goat

2489 Determining the absolute bioavailability of rumen-protected Met using the selenomethionine method in lactating cows. J. Kim^{*1,2}, H. Hu², K. Park^{1,2}, and C. Lee^{1,2}, ¹Interdisciplinary Nutrition Program, Department of Animal Sciences, The Ohio State University, Wooster, OH, ²Department of Animal Sciences, The Ohio State University, Wooster, OH.

The objective of the study was to evaluate the selenomethionine (Se-Met) method to measure absolute bioavailability (BA) of rumen-protected Met (RP-Met). Thirty-six mid-lactating cows were used in a replicated 4 × 4 Latin square design. All cows received a basal diet that met the MP requirement but 83% of the Met requirement for 10 d (basal period) followed by the backgrounding diet (the basal diet with 0.15% Se-yeast; backgrounding period) for 14 d. Then, cows received the experimental diets in a 4 × 4 Latin square with 14-d periods. The experimental diets were the backgrounding diet with 1 of 4 RP-Met (A, B, C, and D; supplying 21 g/d Met). Milk was sampled from 4 consecutive milkings on the last 2 d in each period for Se and N analyses. Fecal samples were collected on the last 3 d in each period (6-time points) to estimate total Se excretion and absorption. The true absorption of Se from Se-Met was obtained by Se absorption during the experimental period corrected for Se absorption during the basal period. The entry of Met in the gut was calculated by absorbed Se from Se-Met divided by milk Se:Met. All data were analyzed using the mixed procedure of SAS with random effects of block, cow within a block, and period and a fixed effect of treatment. Dry matter intake, milk and component yields, and Se excretion in feces

did not differ among treatments. Milk Se:N tended to be greater ($P = 0.09$) for RP-Met B compared with others, suggesting that RP-Met B was potentially less bioavailable. However, calculated Met entries did not differ among treatments. The LSM of the Met entry during the backgrounding and for treatments during the experimental period were used to calculate the absolute BA, which were 75.1%, 89.4%, 100.1%, and 85.1% for RP-Met A, B, C, and D, respectively. In conclusion, although the BA of RP-Met D was comparable to the expected (80%), RP-Met C was overestimated. In addition, the Met entry obtained for cows during the background period was unrealistic when compared with NASEM (145 vs. 56 g/d). Therefore, the Se-Met method is not likely appropriate to obtain absolute BA.

Key Words: feces, Se-yeast, true absorption

2490 Effects of phytogenic supplementation at different protein levels in lactating dairy diets. N. Alfaro-Diaz^{*1}, M. Siregar¹, A. Osorio¹, S. Saikumar¹, G. K. Salas-Solis¹, K. Arriola¹, J. Davidson², T. Aubert², and D. Vyas¹, ¹University of Florida, Gainesville, FL, ²Cardigan Animal Nutrition, Wayzata, MN.

We evaluated the effects of phytogenic supplementation at different protein levels in lactating dairy diets. We hypothesized that phytogenic supplementation with low-protein lactation diets will enhance animal performance comparable to high-protein diets. Twenty-four lactating Holstein cows were used in a 4 × 4 Latin square design, balanced for carry-over effects with four 28-d experimental periods (21 d adaptation + 7 d collection) and 4 treatments arranged in a 2 × 2 factorial (protein level and phytogenic supplementation). Treatments were crude protein level at 16.5% without (CON) or with phytogenic (CONP), and lower crude protein level at 14.5% without (LOW) or with phytogenic (LOWP). Phytogenic supplement was top-dressed (7 g/d) on the TMR. Intake and milk yield were recorded daily, and milk samples were collected during the last week of experimental period for milk composition. Data were analyzed using PROC GLIMMIX of SAS 9.4 and statistical model included main effects of protein, phytogenic supplement, and their interaction and parity and square as a fixed effect, and cow and period as random effect. Significance was declared at $P \leq 0.05$. Milk yield (MY; 40.3 vs. 39.0 ± 2.31 kg; $P = 0.04$) was greater and feed efficiency (MY/DMI; 1.65 vs. 1.57; $P = 0.06$) tended to be greater with high protein diets while no effect was observed with phytogenic supplementation on DMI, MY, and feed efficiency. Milk fat concentration (3.99% vs. 3.83% ± 0.19%; $P = 0.03$) was greater while milk fat yield ($P = 0.06$) and 3.5% fat-corrected milk (FCM) tended to be greater with phytogenic supplementation ($P = 0.09$). The proportion of de novo ($P = 0.02$) and preformed fatty acids ($P = 0.03$) were greater while mixed fatty acids ($P = 0.06$) tended to be greater with phytogenic supplementation. No treatment effects were observed for plasma glucose, and BUN ($P > 0.10$). Overall, phytogenic supplementation, despite protein levels, enhanced FCM, milk fat concentration and yield, as well as the proportion of de novo and preformed fatty acids. Additionally, high protein diets, regardless of phytogenic supplementation, increased milk yield and showed a tendency to improve feed efficiency.

Key Words: phytogenic protein dairy

2491 Effects of iso-butyrate and 2-methyl butyrate with different levels of rumen-degraded protein on ruminal and total tract digestibility and microbial protein synthesis in lactating cows. K. Park^{*1}, K. Clark², J. L. Firkins³, D. Kleinschmit⁴, M. Socha⁴, and C. Lee², ¹Interdisciplinary Nutrition Program, Department of Animal Sciences, The Ohio State University, Wooster, OH, ²Department of Animal

Sciences, The Ohio State University, Wooster, OH, ³Department of Animal Sciences, The Ohio State University, Columbus, OH, ⁴Zinpro Corporation, Eden Prairie, MN.

The study aimed to determine the interactive effects between iso-butyrate and 2-methyl butyrate (IB-2MB) and rumen degraded protein (RDP). Eight rumen-cannulated Holstein cows were used in a replicated 4 × 4 Latin square design with 2 × 2 factorial treatment arrangement: 9% or 11% RDP (dietary DM; NASEM, 2021; 15.3% vs. 17.0% CP; 29.7% starch; 29.2% NDF) with or without IB-2MB (0.09% IB and 0.06% 2MB in dietary DM). The triple marker system with the reticular sampling technique was used to determine ruminal digestibility and microbial protein synthesis. Total collection of feces was conducted to determine total-tract digestibility. Data were analyzed using the PROC MIXED of SAS with RDP, IB-2MB, RDP × IB-2MB, and period as fixed effects, and square and cow within square as random effects. There was no interaction between RDP and IB-2MB on digestibility and microbial protein synthesis. The level of RDP did not affect ruminal true digestibilities of OM and nonammonia N (NAN), but high RDP (HRDP) decreased ruminal NDF digestibility (23.4% vs. 27.3%; $P < 0.01$) compared with low RDP (LRDP). The level of RDP did not affect total-tract digestibility of nutrients except that N digestibility was greater for HRDP (74.2% vs. 71.1%; $P < 0.01$). The supplementation of IB-2MB decreased ruminal NDF (23.4% vs. 27.3%; $P < 0.01$) and true NAN digestibility (71.4% vs. 77.1%; $P \leq 0.03$) and tended to decrease OM digestibility (69.0% vs. 72.3%; $P \leq 0.06$), but total-tract digestibilities were not affected by IB-2MB. While bacterial NAN flows were not affected by RDP or IB-2MB, bacterial NAN per unit of OM truly digested was greater for HRDP versus LRDP (37.0 vs. 34.6 g/kg; $P < 0.01$) and for IB-2MB (36.5 vs. 35.0 g/kg; $P \leq 0.05$). In conclusion, IB-2MB improved the efficiency of microbial protein synthesis but did not improve the digestibility of nutrients. Previously, we reported that IB-2MB increased milk fat yield under HRDP (1.20 vs. 1.43 kg/d; $P < 0.01$). The increased milk fat yield by IB-2MB under HRDP was not due to the improved digestibility.

Key Words: branched-chain volatile fatty acid, isoacid

2492 Effects of rumen-protected amino acids on body fat reserves and protein mobilization in fresh cows. M. M. Efil¹*, F. C. Cardoso¹, L. Bahloul², and J. K. Drackley¹, ¹University of Illinois Urbana-Champaign, Urbana, IL, ²Adisseo France S.A.S, Malicorne, France.

The goal of this experiment was to establish the effect of supplementation of the rumen protected amino acids (RPAA) Met and Lys (Adisseo) to the diet of fresh cows on body fat reserves and protein mobilization. Primiparous ($n = 40$) and multiparous ($n = 48$) Holstein cows were blocked by calving date and lactation number and within blocks assigned randomly to 1 of 4 treatments beginning at calving. During the far-off and close-up dry periods, all cows received the same diets. Treatments were as follows: (1) Control (CON), basal diet AA supply without RPAA, 45 g/d of Met and 137 g/d of Lys (3.06 Lys:Met), (2) MET, supplemental Met, 53 g/d Met and 137 g/d Lys (2.61 Lys:Met), (3) ML, supplemental Met and Lys, 53 g/d Met and 162 g/d Lys (3.04 Lys:Met), and (4) HML, high supplemental Met plus Lys, 62 g/d Met and 162 g/d Lys (2.61 Lys:Met). Cows were on experiment from 24 d before expected calving and treatments continued from calving until 63 d post-calving. Data were analyzed using a randomized block design with the MIXED procedure of SAS (SAS Institute Inc.). The statistical model included treatment, day, and the treatment by day interaction. The DMI was 18.0, 19.1, 19.9, and 19.1 kg/d and energy-corrected milk was 34.7, 36.5, 38.6, and 35.7 kg for CON, M, ML, and HML, respectively.

Images of ultrasounds of back fat thickness and longissimus dorsi depth were obtained above the 12th intercostal space at d 1, 7, 14, 21, 28, and 63 postpartum. Cows fed HML tended ($P = 0.08$) to have higher back fat thickness than CON (0.59 cm vs. 0.48 cm) at d 63. Cows fed M and HML tended ($P = 0.06$ and $P = 0.09$; respectively) to have lower back fat loss (0.14 cm and 0.19 cm, respectively) than the cows fed ML (0.66 cm) between d 1 and 63. Cows fed CON had the lowest longissimus dorsi depth at d 63 (3.57 cm; $P < 0.001$). The lowest loss for longissimus dorsi depth was for cows fed HML between d 1 and 63 (0.85 cm; $P = 0.02$). Addition of RPMet and RPLys to fresh cow diet had a positive effect to prevent the loss at body fat reserves and longissimus muscle depth, except for the combination of high Met and a low Lys:Met.

Key Words: methionine, lysine, transition cow

2493 Impacts of altering rumen-degradable to rumen-undegradable protein ratio on milk yield and components, feed efficiency, and rumen metabolome in lactating dairy cows. M. Suazo^{*}, J. Stypinski, J. Felton, and I. Salfer, University of Minnesota, Saint Paul, MN.

The objective of this experiment was to assess effects of dietary RDP:RUP ratio achieved through increasing inclusion of soybean meal (SBM) on milk synthesis and rumen metabolome. Twelve lactating Holstein cows (177 ± 53 ; DIM $\pm$ SD) were enrolled in a 4 × 4 Latin-square design ($n = 12$ cows/treatment). Cows were assigned to 1 of 4 treatments including: RDP:RUP ratios of 50:50 (RUP50), 55:45 (RUP45), 60:40 (RUP40), and 65:35 (RUP35). Diets were formulated to be isonitrogenous at 16.4% CP. The RDP:RUP ratio was altered by substituting SBM with alfalfa hay and whole cottonseed, while adding soy hulls and balancing corn and corn silage to maintain NDF and starch concentrations across treatments. Periods consisted of 18 d of adaptation and 3 d of sampling. Milk yield (MY) and DMI were determined daily throughout the experiment. Milk samples analyzed for fat, protein, lactose, and MUN concentration and rumen fluid was collected via oral tube for metabolome analysis. Data were analyzed as a mixed model with the fixed effects of RDP:RUP ratio and random effects of period, day nested within period, and cow, with linear and quadratic orthogonal contrasts. Milk yield was affected by treatment ($P = 0.04$), with RUP50 (29.1 kg/d) having lower MY than the RUP45 (30.3), RUP40 (30.7) and RUP35 (30.4; SE = 1.67; $P < 0.04$). Similarly, fat yield was linearly decreased by increased RUP ($P < 0.001$) with yields of 1,371, 1,334, 1,304, and 1,235 g/d for RUP35, RUP40, RUP45, and RUP50, respectively (SE = 55.5). Milk protein yield was not affected by treatment ($\mu = 1,085$ g/d; SE = 44.3; $P = 0.28$). Dry matter intake was quadratically affected by treatment, with RUP35 (24.0 kg/d) lower than other treatments and RUP50 (25.1) lower than RUP40 (25.8) and RUP45 (26.9; SE = 0.55; $P < 0.001$). Milk urea nitrogen increased linearly with decreased RDP ($P < 0.001$, SE = 0.54) from 11.0 mg/dL in RUP35 to 18.9 mg/dL in RUP50. Milk/DMI was affected by RDP:RUP ($P < 0.001$), with greatest feed efficiency in RUP35 (1.29 kg/kg) and lowest in RUP45 (1.14; SE = 0.067). Overall, somewhat surprisingly, increasing RUP decreased milk yield and feed efficiency compared with greater RDP.

Key Words: soybean meal, RDP, RUP

2494 Effects of iso-acids and dietary protein concentration on total tract digestibility and urine purine derivatives of lactating dairy cows. M. Suazo^{*}1, M. T. Socha², D. Kleinschmit², and I. Salfer¹, ¹University of Minnesota, Saint Paul, MN, ²Zinpro Corporation, Eden Prairie, MN.

Iso-acids are 4- to 5-carbon-chain VFA that can increase growth of cellulolytic bacteria by providing carbons to be used for microbial protein and fatty acid synthesis, increasing fiber digestibility. The objective of this experiment was to determine the effect of IA in low and moderate CP diets on total-tract digestibility (TTD) and markers of microbial protein synthesis. Twelve cannulated late-lactation multiparous Holstein cows (210 ± 40.3 ; DIM $\pm$ SD) were enrolled in a 4×4 Latin square design ($n = 12$ cows/treatment). Treatments were arranged as a 2×2 factorial design with a TMR formulated for either 15% CP diet (LO) or 16.5% CP diet (MOD), without (CON) or with 40 g of an isoacid blend (IA). Treatments were LO-CON, LO-IA, MOD-CON, and MOD-IA. Periods consisted of 4 wk of adaptation and 1 wk of sampling. The TMR, refusal, individual ingredients, and concentrates were taken weekly throughout the experiment. Fecal and urine samples were taken 4 $\times$ over the last 2 d of each period and composited by period. Dried (55°C for 48 h) feed and fecal samples were analyzed for CP, starch, and NDF. Urine was acidified upon collection and analyzed for purine derivative (creatinine and allantoin) concentrations. The TTD of CP was increased by increased dietary CP (74.4% vs. 70.1%; SE = 0.4; $P < 0.01$) and by IA inclusion (72.6% vs 71.9%; SE = 0.4; $P < 0.01$). The TTD of NDF was greater for LO-IA vs. all other treatments ($P = 0.02$). Starch digestibility was affected by the interaction of CP $\times$ IA (SE = 0.81; $P = 0.02$), with LO-IA having a greater digestibility than LO-CON and MOD-IA. Allantoin concentration surprisingly tended to decrease by 14% with increased dietary CP ($P = 0.06$) and MOD-IA tended to have a 16%, 17%, and 11% lower concentrations than LO-CON, LO-IA and MOD-CON, respectively (SE = 0.23; $P < 0.06$). Similarly, creatinine tended to be 10% lower with the higher protein diet ($P = 0.08$) and MOD-IA was 19% greater than LO-CON (SE = 0.05; $P = 0.04$). Iso-acids appeared to improve TTD of protein and improved TTD of NDF but were dependent on CP level.

Key Words: branched-chain VFA (BCVFA), total tract NDF digestibility (TTNDFD), microbial protein synthesis

2495 Comparison between 2 different sources of rumen-undegradable protein in the diet of lactating cows and their impact on productive performance. A. C. Mocelin¹, M. M. Marquetti¹, N. P. G. Viana¹, L. C. Obialeski¹, L. C. Copetti^{1,2}, M. Bugoni¹, B. J. A. Villar², J. H. Carneiro², and R. Almeida^{*1}, ¹Universidade Federal do Paraná, Curitiba, PR, Brazil, ²Dairy Inside Consultoria, Castro, PR, Brazil.

The objective of this study was to compare 2 different sources of rumen undegradable protein (RUP) in the diet of Holstein cows and their impact on milk production and composition. A total of 288 cows (133 primiparous and 155 parous), with 142 ± 84 DIM and producing 49.9 ± 10.9 kg/d, were enrolled in a trial conducted in a commercial herd in the state of Paraná, Southern Brazil, from September to November 2024. The animals were blocked by parity, DIM, and milk production in a crossover experimental design, lasting 35 d each experimental period. The experimental diets provided were isoproteic (17.1%CP, 10.0%MP, and 6.1%RUP), with soybean meal partially replaced by 6% protected soybean meal (PSBM) or high-protein dried distillers grains (HPDDG). Milk yield was recorded daily and milk samples were collected in the last 3 d of each period, over 9 consecutive milkings. Data were analyzed using the MIXED procedure of SAS, considering the fixed effects of period, parity, treatment, time, and their interactions, and the random effects of block and cow within period. Cows from the HPDDG group produced more milk ($P = 0.048$) than the PSBM group (50.27 vs. 49.15 ± 0.40 kg/d). However, the HPDDG group had lower milk fat content ($P < 0.01$) and fat yield ($P = 0.02$) than PSBM (3.36% vs. $3.58\% \pm 0.04\%$ and 1.66 vs. 1.74 ± 0.023 kg/d, respectively). There were no differences

($P > 0.10$) in 3.5%FCM and ECM yields, and milk protein and lactose contents and yields between treatments. Milk urea nitrogen was lower ($P < 0.01$) in the HPDDG group compared with PSBM (12.54 vs. 14.54 ± 0.17 mg/dL). Saturated FA was higher ($P < 0.01$) in the PSBM group compared with HPDDG (2.13 vs. 1.96 g/100 g milk). De novo FA was also higher ($P < 0.01$) for PSBM group compared with the HPDDG (0.81 vs. 0.74 g/100 g milk and 22.6 vs. 22.2 g/100 g FA), whereas preformed FA was higher ($P < 0.01$) in the HPDDG group compared with PSBM (35.6 vs. 34.0 g/100 g FA). Cows treated with HPDDG produced more fluid milk, but with lower milk fat content and yield, with lower de novo FA and higher preformed FA, compared with cows treated with PSBM.

Key Words: dried distillers grain, protected soybean meal

2496 Effects of rumen-protected methionine on production performance and gut epithelium function during a heat-stress challenge in lactating Holstein cows. J. Mauck¹, H. Pitts^{*1}, M. Usman¹, N. Unnawong², A. Cherdthong², N. Wichasit³, J. Guyader⁴, T. Fernandes⁵, F. C. Cardoso¹, B. R. Loman¹, D. Nolan¹, and J. J. Loo¹, ¹University of Illinois, Urbana, IL, ²Khon Kaen University, Khon Kaen, Thailand, ³Naresuan University, Phitsanulok, Thailand, ⁴Evonik Operations GmbH, Hanau-Wolfgang, Germany, ⁵Virginia Tech, Blacksburg, VA.

Heat stress (HS) reduces production performance and can damage ruminal and small intestinal epithelium, compromising nutrient absorption. Previous HS challenge of dairy cows in environmentally-controlled chambers reported that damage of the ruminal epithelium was associated with alterations in genes involved in Met metabolism. The present study investigated the effects of rumen-protected Met (RPM, Mepron, Evonik Operations GmbH) before and during an HS challenge using electric heat blankets (EHB) on production performance and ruminal and intestinal epithelium function. After a 7-d adaptation on a basal diet without RPM, 11 lactating multiparous Holstein cows (711 ± 28 kg BW; 28 ± 2.0 kg milk/d) were assigned to a control (no RPM, $n = 5$) or an RPM-supplemented diet (0.10% of diet DM; RPM, $n = 6$) for 31 d including a HS challenge with EHB during the last 10 d. After completion of the last day of HS, cows were euthanized via captive bolt for collection of ruminal and intestinal tissue. Gut barrier function and active nutrient transport were determined (ohms/ 0.5 cm^2) in Ussing chambers via transepithelial electric resistance (TEER) and changes in transepithelial short-circuit current (Physiologic Instruments, Nevada). Data were analyzed using PROC MIXED in SAS. Overall respiration rate during HS was greater ($P < 0.01$) in cows fed RPM, but body and vaginal temperature did not differ. Before HS, feeding RPM led to greater ($P < 0.05$) energy-corrected-milk (ECM, 35 vs. 29 kg/d), milk protein % (3.61 vs. 3.32) and yield (1.02 vs. 0.81 kg/d). The DMI tended ($P = 0.07$) to be greater with RPM (28.0 vs. 24.2 kg/d). Regardless of treatment, milk yield decreased ($P < 0.01$) by $\sim 9\%$ during the HS challenge but DMI was not affected. However, ECM tended ($P = 0.07$) to be greater and milk casein % (2.91 vs. 2.72) and milk protein yield (1.13 vs. 1.01 kg/d) were greater ($P < 0.05$) due to RPM. The TEER in ruminal epithelium was greater ($P = 0.05$) when feeding RPM. Peptide (Gly-Sar) and free Met transport also were greater ($P \leq 0.05$) in cows fed RPM. Overall, RPM enhanced production performance before and during HS and prevented decreases in ruminal and intestinal epithelium function.

Key Words: amino acid, milk protein, rumen

2497 Rumen-protected methionine, guanidinoacetic acid, and their effects on production performance and plasma biomarkers during a heat-stress challenge in lactating Holstein cows. J. Mauck¹, H. Pitts^{*1}, M. Usman¹, N. Unnawong², A. Cherdthong², N.

Wichasit³, J. Guyader⁴, T. Fernandes⁵, F. C. Cardoso¹, D. Nolan¹, and J. J. Loo¹, ¹University of Illinois, Urbana, IL, ²Khon Kaen University, Khon Kaen, Thailand, ³Naresuan University, Phitsanulok, Thailand, ⁴Evonik Operations GmbH, Hanau-Wolfgang, Germany, ⁵Virginia Tech, Blacksburg, VA.

Heat stress (HS) is a significant challenge for the dairy industry, often causing serious health and production consequences for the dairy cow. Nutrition may help maintain metabolism and production during periods of HS. We investigated effects of rumen-protected methionine (RPM, Mepron, Evonik Operations GmbH) and guanidinoacetic acid (GAA, GuanAMINO, Evonik Operations GmbH) during a HS challenge using electric heat blankets (EHB) on production performance, plasma biomarkers, and ruminal VFA. Ten multiparous Holstein cows (5 with ruminal cannulas) were used in a 5 × 5 Latin square. Each period lasted 23 d with 16 d of thermoneutral feeding of the assigned diets followed by 7 d of HS using EHB. Treatments were: no HS (CON-TN); 7 d of HS (CON-HS); 7 d of HS with RPM (0.10% of diet DM; RPM-HS); 7 d of HS with GAA (GAA-HS, 25 g/d); and 7 d of HS with RPM+GAA (MIX-HS). Data were analyzed using PROC MIXED in SAS. Pre-planned contrasts were: CON-HS vs. CON-TN, CON-HS vs. RPM-HS, CON-HS vs. GAA-HS, and RPM-HS vs. GAA-HS. Both rectal and vaginal temperature (+0.33°C) and respiration rate (+11 breaths/min) were greater ($P < 0.01$) in CON-HS vs. CON-TN. None of the dietary treatments influenced these responses. Although DMI was 3.9 kg/d lower ($P = 0.01$) in CON-HS vs. CON-TN, milk yield was not affected. Neither RPM nor GAA affected these responses. Compared with CON-TN, feeding CON-HS led to lower milk protein % (2.99% vs. 3.21% ± 0.08%), but cows fed RPM had greater ($P = 0.001$) milk protein % (3.27% vs. 2.99% ± 0.08) relative to CON-HS. This response resulted in greater ($P = 0.02$) milk protein yield in cows fed RPM vs. CON-HS. Except for greater ($P = 0.04$) concentrations of isovalerate and isobutyrate in ruminal fluid from cows fed CON-HS vs. CON-TN, there were no other treatment effects on individual or total VFA. Among the plasma biomarkers analyzed, concentration of aspartate aminotransferase (61.9 vs. 70.3 ± 6.0 U/L) was lower in RPM-HS vs. CON-HS. Overall, the lower DMI and greater body temperature indicate that the EHB was effective in stressing the cows. Although RPM did not affect performance, it was effective in preventing decreases in milk protein and the negative impact of HS on liver function.

Key Words: amino acid, lactation, milk protein

2498 Ruminal papillae mRNA abundance of metabolic, antioxidant, and barrier function pathways during a heat-stress challenge in lactating Holstein cows fed rumen-protected methionine and guanidinoacetic acid. J. Mauck¹, N. Unnawong², H. Pitts^{1*}, F. Li³, N. Wichasit⁴, A. Cherdthong², J. Guyader⁵, F. Cardoso¹, D. Nolan¹, and J. J. Loo¹, ¹University of Illinois, Urbana, IL, ²Khon Kaen University, Khon Kaen, Thailand, ³Jilin University, Changchun, China, ⁴Naresuan University, Phitsanulok, Thailand, ⁵Evonik Operations GmbH, Hanau-Wolfgang, Germany.

Heat stress (HS) reduces feed intake and diverts blood flow from the core of the body to the periphery, causing hypoxia in the digestive tract epithelium and altering tight junctions, immune, metabolic, and nutrient transport pathways. Nutrition may reduce detrimental effects of HS on cellular pathways associated with proper gut epithelium function. We investigated the effects of rumen-protected methionine (RPM; Mepron, Evonik Operations GmbH) and guanidinoacetic acid (GAA; GuanAMINO, Evonik Operations GmbH) during a HS challenge using electric heat blankets (EHB) on ruminal papillae mRNA abundance. Ten multiparous Holstein cows (5 with ruminal cannulas) were used in a 5

× 5 Latin square. Each period lasted 23 d with 16 d of thermoneutral feeding of the assigned diets followed by 7 d of HS using EHB. Treatments were no HS (CON-TN); 7 d of HS (CON-HS); 7 d of HS with RPM (0.10% of diet DM; RPM-HS); 7 d of HS with GAA (GAA-HS, 25 g/d); and 7 d of HS with RPM+GAA (MIX-HS). Data were analyzed using PROC MIXED in SAS. Preplanned contrasts were CON-TN vs. CON-HS, CON-HS vs. RPM-HS, CON-HS vs. MIX-HS, and RPM-HS vs. GAA-HS. Ruminal papillae were used for RNA extraction and quantitative RT-PCR of 30 target genes. Rectal and vaginal temperature (+0.33°C) and respiration rate (+11 breaths/min) were greater ($P < 0.01$) in CON-HS vs. CON-TN, but RPM, MIX, or GAA had no effect. Dry matter intake was 3.9 kg/d lower ($P = 0.01$) in CON-HS vs. CON-TN, but neither RPM, MIX, nor GAA impacted these responses or milk yield. In addition to greater ($P < 0.05$) milk protein percentage and yield in cows fed RPM vs. CON-HS, they had greater papillae abundance of genes associated with antioxidant pathways generating glutathione, taurine, and polyamines (*CBS*, *SMS*, *ODC1*). Tight junction protein 1, *GSTT4*, *HMOX1*, and *TYMS* also were upregulated ($P < 0.05$), while some amino acid transporters were downregulated in RPM vs. CON-HS. Feeding MIX led to similar responses to RPM. Although markers of cellular heat stress (*HSPA5*) and inflammation (*TLR4*) were not affected by HS, RPM, or MIX, the observed changes suggested important adaptations in tissue junctional complexes, antioxidant synthesis, and control of reactive oxygen species during a HS challenge.

Key Words: gene expression, metabolism, nutrition

2499 Rumen-protected methionine and its effects on ruminal microbiota during a heat-stress challenge in lactating Holstein cows. Q. Jiang¹, F. Li^{2,3}, H. Pitts^{3*}, J. Mauck³, M. Usman³, N. Unnawong⁴, A. Cherdthong⁴, N. Wichasit⁵, J. Guyader⁶, T. Fernandes⁷, F. C. Cardoso³, D. Nolan³, and J. J. Loo³, ¹University of Illinois, Chicago, IL, ²Jilin University, Changchun, China, ³University of Illinois, Urbana, IL, ⁴Khon Kaen University, Khon Kaen, Thailand, ⁵Naresuan University, Phitsanulok, Thailand, ⁶Evonik Operations GmbH, Hanau-Wolfgang, Germany, ⁷Virginia Tech, Blacksburg, VA.

Microbial communities in the rumen are dynamic and respond to external factors such as heat stress (HS). Our previous work with lactating cows fed rumen-protected methionine (RPM) identified alterations in the abundance of several bacteria likely resulting from the greater DMI with RPM. We investigated effects of RPM (Mepron, Evonik Operations GmbH) before and during a HS challenge using electric heat blankets (EHB) on the ruminal microbiota. After a 7-d adaptation on a basal diet without RPM, 11 multiparous Holstein cows (711 ± 28 kg BW; 28 ± 2.0 kg milk/d) were assigned to a control (no RPM, n = 5) or RPM diet (0.10% of diet DM; RPM, n = 6) for 31 d including a HS challenge with EHB during the last 10 d. Cows were then euthanized via captive bolt for collection of ruminal digesta. DNA extracted from digesta was subjected to PacBio Full-Length 16S rRNA gene sequencing. High-quality amplicon sequence analyses were performed with Targeted Amplicon Diversity Analysis (TADA), MicrobiomeAnalyst, PICRUST2, and STAMP software. Production data were analyzed using PROC MIXED in SAS. Before HS, RPM-fed cows had greater ($P = 0.05$) energy-corrected milk ($P = 0.05$) and tended ($P = 0.07$) to have greater DMI. Regardless of diet, milk yield decreased ($P < 0.01$) by ~9% during HS challenge but DMI was not affected. Despite similar DMI (25.4 ± 1.5 kg/d), ruminal acetate:propionate was greater ($P = 0.02$) due to RPM. In addition, ruminal microbiota in the RPM group had a lower Shannon index ($P = 0.06$), but greater abundance of the *Eubacteriaceae* and *Synergistales* family, suggesting benefits in amino acid and carbohydrate fermentation. In contrast, aldehyde dehydrogenase

(NAD⁺), guanine-methyltransferase, and methionine gamma-lyase were among microbial enzymes underrepresented ($P < 0.05$) in response to RPM. Several predicted microbial fatty acid synthesis pathways also were lower ($P = 0.05$) with RPM, including oleate biosynthesis IV and palmitoleate biosynthesis I. In contrast, the predicted superpathway of L-threonine metabolism, leading to propionate synthesis, and glycerol degradation to butanol were upregulated ($P < 0.05$) by RPM. Besides its positive effects on energy-corrected milk yield before HS, it is possible that the tendency for greater DMI with RPM had an impact on the microbiota.

Key Words: amino acid, microbiome, digestion

2500 Assessing the impact of oscillating dietary crude protein on the stability of the rumen microbiome in dairy cattle. F. Viquez-Umana¹, M. Erickson³, J. Young², G. I. Zanton², M. A. Wattiaux¹, G. Suen¹, and H. Mantovani^{*1}, ¹University of Wisconsin–Madison, Madison, WI, ²US Dairy Forage Research Center, Madison, WI, ³University of Minnesota, Saint Paul, MN.

Understanding how the rumen microbiota responds to varying protein levels and feeding patterns is critical for optimizing dairy cattle nutrition. This study investigated the influence of dietary crude protein (CP) levels (13.8% or 15.5% CP of ration dry matter) and CP feeding patterns (constant over time [static] or oscillating by 1.8 percentage units above and below the mean every 48 h) on the composition, diversity, and function of the rumen microbiome. Using a replicated Latin square design, 8 rumen-cannulated Holstein cows were assigned each of the 4 dietary treatments (structured as a 2 × 2 factorial) in 4 consecutive 28-d periods (with 24 d of adaptation and 4 d of sampling). Rumen samples were collected 4-h post-feeding, and amplicon libraries of the V4 region of the 16S rRNA gene were sequenced and analyzed to assess changes in microbiome composition. Additionally, volatile fatty acids (VFAs) were measured to evaluate rumen microbial function. Results revealed that oscillating diets increased rumen microbial diversity (Shannon index, $P = 0.04$). The rumen microbiome composition was also affected by CP feeding pattern ($P = 0.05$), but not by dietary CP level ($P = 0.21$). Furthermore, differential abundance analysis using ANCOM-BC identified the genera CAG-352 ($P = 0.0001$) and an unclassified member of the family *Acholeplasmataceae* ($P = 0.0002$) as significantly affected by protein level and feeding pattern. Last, VFA profiles remained consistent across treatments, with no observable changes in relative concentration. These findings support the hypothesis that the rumen microbiome exhibits stability even under substantial variation in ruminal supply of dietary CP, indicating the presence of compensatory mechanisms. These results emphasize the importance of further research into rumen metabolic processes and host-microbiome interactions to unlock new opportunities for enhancing protein nutrition in dairy cattle.

Key Words: amplicon sequencing, microbiome stability, dietary protein

2501 Evaluation of a rumen-protected methionine product in lactating dairy cows supplemented in an automatic milking system. C. M. Harris^{*1}, A. L. Carroll¹, E. Neto², J. Vasconcelos², J. Hergenreder², F. Sun², and P. J. Kononoff¹, ¹Department of Animal Science University of Nebraska–Lincoln, Lincoln, NE, ²Kemin Industries, Inc., Des Moines, IA.

Methionine is a critical amino acid for lactating dairy cows, essential for milk protein synthesis, and often supplemented as rumen-protected methionine (RPM). This study evaluated the effects of feeding a commercial RPM product (KESSENT Me, Kemin Industries Inc., Des

Moines, IA) on milk protein yield in lactating Holstein cows using an automatic milking system (AMS). Fifty-one cows (16 primiparous [PRIMI]; 35 multiparous [MULTI]) were randomly assigned to control (no RPM) or treatment groups. Cows were offered a daily allotment of concentrate (meal form) within the AMS, where each cow received one of the 2 treatments. On wk 4 of lactation, cows began to receive the treatments for 14 weeks (98 d). The control concentrate contained 0.24% rumen-protected lysine (RPL; LysiGEM Extend, Kemin Industries, Inc., Des Moines, IA), 34.0% expellers soybean meal, 65.4% ground corn, 0.41% molasses, and no RPM. The treatment concentrate contained 0.24% RPL, 33.7% expeller soybean meal, 65.4% ground corn, 0.41% molasses, and 0.28% test RPM. Data were analyzed as a randomized complete block using proc MIXED of SAS (version 9.4, SAS Institute Inc., Cary, NC). Fixed effects included treatment, parity (PAR), week, and their interactions, and block was random. An interaction ($P = 0.02$) was observed between PAR and RPM on milk protein yield. Milk protein yield increased in MULTI cows from 1.33 to 1.36 ± 0.036 kg/d but reduced in PRIMI cows (1.33 to 1.16 ± 0.056 kg/d). The percent of milk protein was not different ($P > 0.20$) by PAR, treatment, or interaction, averaging 3.08% ± 0.057%. An interaction ($P = 0.05$) between treatment and PAR was observed on milk yield. Milk yield was similar in MULTI cows, averaging 44.0 kg/d ± 1.18 kg/d, but in PRIMI cows, feeding RPM reduced milk yield from 43.6 to 38.8 ± 1.82 kg/d. The percentage (3.78% ± 0.180%) and yield (1.60 ± 0.083 kg) of milk fat were not affected by treatment ($P > 0.13$), and no interaction ($P > 0.36$) between PAR and treatment was observed. The study suggests RPM supplementation can enhance milk protein yield in MULTI cows under commercially relevant conditions.

Key Words: methionine, automatic milking system

2502 Effects of rumen-protected histidine on performance of early-lactation Holstein cows. C. V. Almeida^{*1}, L. F. Martins¹, S. Issa-bekova¹, S. F. Cueva², C. J. Eifert¹, A. Richards¹, N. Stepanchenko¹, D. E. Wasson¹, and A. N. Hristov¹, ¹The Pennsylvania State University, University Park, PA, ²Cargill Animal Nutrition, Shippensburg, PA.

Few studies have evaluated the effects of rumen-protected (RP) His as a limiting amino acid in early lactation cows. Therefore, the aim of this study was to evaluate the effects of feeding RPHis on lactational performance of Holstein cows during the first 80 DIM. A randomized complete block design experiment was conducted with 40 cows, including 16 primiparous and 24 multiparous. Cows were blocked by parity, calving date, and milk predicted transmitting ability for primiparous or by lactation number and previous lactation first 100 DIM milk production for multiparous cows. Experimental treatments (TRT) were: (1) control (CON) diet, supplemented with RPLys RPMet, and (2) the CON diet supplemented with RPHis. The CON diet was designed to supply MP at 95% of NASEM (2021) requirements calculated based on production data collected at the start of the experiment. However, based on production and DMI data throughout the experiment, the basal diet supplied MP at 103% of requirements and CON was only slightly deficient in mHis (108% of target mHis efficiency, according to NASEM, 2021). Data were analyzed using the MIXED procedure of SAS as repeated measures with treatment (TRT), week, parity, and TRT × wk and TRT × parity interactions in the model. Overall milk yield (37.9 ± 2.3 kg/d), DMI (22.4 ± 1.1 kg/d), BW (632 ± 19 kg) and BW change (−0.626 ± 0.21 kg/d) of the cows were not affected by TRT. Milk fat and protein concentrations (4.50% ± 0.118% and 3.09% ± 0.068%) and yields (1.68 ± 0.115 and 1.16 ± 0.065 kg/d, respectively) were also not affected by TRT. Plasma concentration of His was increased ($P < 0.001$) by 16% by RPHis, compared with CON. There were no other effects of TRT on

plasma AA, except an increase in Pro by RPHis. There was a TRT × week interaction ($P \leq 0.05$) for plasma concentrations of His, Phe, Tyr, and 3-methylhistidine. Cows fed RPHis had higher blood concentrations of Phe and Tyr on wk 2, higher His on wk 4, 8, and 10, and lower 3-methylhistidine on wk 1 and 2, but higher on wk 8, compared with CON. In this experiment, performance of early-lactation dairy cows was not affected by RPHis, likely due to oversupply of MP by the basal diet.

Key Words: histidine, early lactation, dairy cow

2503 Effect of bioactive peptide supplementation enriched with minerals on intake, fat-corrected milk yield, and metabolic parameters of primiparous Holstein cows. R. M. da Rosa, A. C. T. Rodrigues, L. S. Lopes, W. S. da Rosa, R. Silveira, L. Marins, M. T. Vieira, L. S. Santos, R. C. Fiori, T. C. da Silva, U. S. Londero, E. R. Komninou, V. R. Rabassa, F. A. B. Del Pino, M. N. Corrêa*, *Federal University of Pelotas (UFPEL), Teaching, Research, and Extension Center in Livestock (NUPEEC HUB), Pelotas, Rio Grande do Sul, Brazil.*

This study aimed to evaluate the effects of including bioactive peptides containing minerals in the diet of primiparous Holstein cows on dry matter intake (DMI), fat-corrected milk yield (FCMY), and metabolic parameters. The experiment was conducted on a commercial farm in southern Brazil, involving 37 primiparous cows between 60 and 140 DIM, randomly assigned to 2 homogeneous groups: treatment group (TG, receiving the Inmilk additive – Inbra Nutrição Animal, Jaguariúna, Brazil; $n = 18$) and control group (CG, without additive; $n = 19$). The additive, composed of lysine, methionine, threonine, zinc, phosphorus, and manganese, was provided at a dose of 1 g/kg of dry matter (DM) consumed from the total mixed ration (TMR), totaling 26 g/animal per day. Dry matter intake was monitored using electronic feed bunks (Ponta Agro, Betim, Brazil), while milk production was measured using the ALPRO milking system (DeLaval, Tumba, Sweden) and corrected to 3.5% fat using the formula: $\text{FCMY } 3.5\% = (0.4255 \times \text{kg of milk}) + [16.425 \times (\% \text{ fat}/100) \times \text{kg of milk}]$. Blood levels of glucose, BHB, and nonesterified fatty acids (NEFA) were analyzed on d 0, 7, 14, 21, 28, 35, 42, 60, 75, and 90 of the experiment. Statistical analysis was performed using the PROC MIXED procedure in JMP Pro 17, considering group, sampling days, and their interactions as fixed effects, and the animal as a random effect. The significance level was set at $P < 0.05$. Cows in the TG showed significantly higher DMI ($P < 0.001$) and FCMY ($P < 0.001$) compared with the CG (DMI: 23.22 ± 0.24 vs. 19.44 ± 0.19 kg/day; FCMY: 34.6 ± 0.4 vs. 31.4 ± 0.4 kg/day). However, no differences were observed between groups for metabolic parameters (glucose: 55.30 ± 0.95 vs. 54.87 ± 0.89 mg/dL, $P = 0.72$; BHB: 0.45 ± 0.016 vs. 0.46 ± 0.015 mmol/L, $P = 0.67$; NEFA: 0.23 ± 0.009 vs. 0.23 ± 0.007 mmol/L, $P = 0.67$). In conclusion, supplementation with bioactive peptides containing minerals increased DMI and FCMY without altering the metabolic profile of primiparous cows.

Key Words: amino acid supplementation, lactation performance, ruminant nutrition.

2504 Effects of 2 rumen-protected methionine products on milk production, composition, and fatty acids profile in lactating Holstein cows. N. P. G. Viana^{1,2}, L. C. Obialeski¹, J. T. R. Carvalho¹, L. S. Nogueira¹, F. H. Dalmass², L. B. Los², T. L. Resende³, D. P. D. Lanna⁴, and R. Almeida^{*1}, ¹Universidade Federal do Paraná, Curitiba, PR, Brazil, ²Frisia Cooperativa Agroindustrial, Carambei, PR, Brazil, ³Evonik Brasil, São Paulo, SP, Brazil, ⁴Universidade de São Paulo, Piracicaba, SP, Brazil.

The aim of this study was to evaluate the effects of 2 rumen-protected methionine (RPM) products on milk yield (MY), composition, and fatty acids (FA) profile in lactating cows. A total of 278 multiparous Holstein cows (185 ± 129 DIM) were grouped by parity, MY, and DIM and randomly assigned to one of 3 treatments; 120 cows supplemented with MET_pH (Smartamine M, Adisseo Inc., France), 116 cows with MET_ethyl (Mepron, Evonik Industries, Germany), and 42 control cows (CT). In a randomized block design, the treatment consisted of daily supplementation of 15 g of RPM (MET_pH or MET_ethyl) mixed with 85 g of corn meal provided top-dressed and divided into 2 daily feedings. The total experimental period was 33 d, with milk samples collected during the first 3 d and the last 3 d (9 consecutive milkings) of the experimental period. An additional milk collection was performed for the analysis of the milk FA, using gas chromatography. Data were analyzed using the SAS MIXED procedure, including the fixed effects of treatment, time, and their interaction, the covariate, and the random effects of block and cow within treatment. There was no difference in MY (40.54 vs. 39.91 kg/d; $P = 0.62$) between MET_pH and MET_ethyl treatments, but both groups treated with RPM showed higher MY (40.23 vs. 37.75 kg/d; $P < 0.01$) and higher milk solids (5.47 vs. 5.01 kg/d; $P < 0.01$) than CT. Cows supplemented with RPM also showed higher milk protein content (3.56% vs. 3.51%; $P < 0.01$), but with no differences for milk fat content (4.35% vs. 4.24%; $P = 0.15$) than CT. Regarding the FA, the RPM group showed higher SFA (72.22% vs. 69.58%; $P < 0.01$), but lower UFA (27.66 vs. 30.35; $P < 0.01$) than CT. Cows supplemented with RPM showed higher de novo FA (26.61 vs. 24.24 g/100g FA; $P < 0.01$), lower preformed FA (36.00 vs. 39.44; $P < 0.01$) and higher odd- and branched-chain FA (3.67 vs. 3.32; $P < 0.01$) than CT. Our results suggest that there were no detectable differences in parameters measured between the 2 products, and both groups supplemented with RPM showed higher milk yield, along with milk solids, and milk fat with a higher de novo FA profile.

Key Words: amino acid, milk fat

2505 Impact of balanced essential amino acids on milk production and composition in early lactating dairy cows. F. Sosa^{*1}, B. Castro¹, B. E. Chasi¹, E. Anta Galvan¹, E. Moreno¹, A. Larsen¹, E. French², J. McClure², M. S. Ortega¹, and S. Arriola Apelo¹, ¹University of Wisconsin–Madison, Madison, Wisconsin, ²USDA Dairy Forage Research Center, Madison, Wisconsin.

Essential amino acids (AAs) are critical for lactating dairy cows' health and productive and reproductive performance, as they cannot be synthesized by the animal and must be provided through the diet. This study aimed to evaluate the impact of balancing essential amino acids on milk production and composition. It was hypothesized that providing balanced AAs would increase milk production and positively influence key milk traits. A total of 85 late-pregnant multiparous Holstein cows were blocked by pregnancy (2nd or higher) and calving date. At calving, cows were randomly assigned to either a high rumen degradable protein (RDP; 12%), low digestible rumen undegradable protein (DRUP; 3.8%) diet, (HRDP) or a low RDP (10.2%), high DRUP diet, balanced for metabolizable His, Leu, Lys, and Met (HMAA). Both diets contained 22.8% forage neutral detergent fiber, 25.6% starch, and 1.79 Mcal/kg dry matter of net energy for lactation. Experimental diets were fed from calving until wk 8 of lactation. Cows were housed in tiestalls and milked 3 times daily. Offered and refused feed was measured daily, and forage dry matter was measured weekly from daily compiled samples. Milk yield was measured daily at each milking, and milk samples for FT-MIR analysis were collected weekly from 6 consecutive milkings. Data were analyzed with a mixed linear model containing lactation, block, week,

and diet as fixed effects, and cow within block and lactation as random. From wk 2–8, cows fed the HRDP diet had higher dry matter intake (DMI), and from wk 3–8 produced more milk than HMAA-fed cows ($P < 0.01$). Despite the observed differences in DMI and milk production, there were no differences in milk true protein and fat yield (diet and week $\times$ diet $P > 0.05$). In conclusion, despite the lower DMI in HMAA cows, they were able to maintain similar yield of milk components, including fat and protein, compared with the HRDP group. These findings suggest that reducing RDP while balancing for key essential AA may enhance nutrient efficiency and reduce the environmental footprint of dairy production.

Key Words: amino acid, early lactation, milk components

2506 Influence of amino acids and energy substrates on milk fat synthesis: A meta-regression analysis. D. Salas* and I. A. M. A. Teixeira, *University of Idaho, Twin Falls, ID.*

In recent years, milk fat prices have risen, approximately 28% higher than the value of milk protein. This increase reflects broader market dynamics, including supply chain constraints, global dairy trade fluctuations, and production costs. Given its economic importance, dairy producers seek nutritional strategies to enhance milk fat production, prompting further research into dietary interventions, such as amino acid supplementation. Although prior studies have linked amino acid supplementation to milk fat synthesis, responses remain highly variable, suggesting that additional dietary factors modulate these effects. This study aimed to identify key dietary components influencing milk fat responses in dairy cows supplemented with rumen-protected methionine (RPM). We hypothesized that variations in nutrient intake, particularly energy substrates and amino acid availability modulate milk fat synthesis in methionine-supplemented cows. To test this hypothesis, we conducted a meta-regression analysis using a database with 239 treatment means from 59 peer-reviewed publications spanning 2004 to 2024. Studies were identified from 3 indexed databases using the search terms “milk fat,” “amino acid,” and “cow,” and were included if they reported production parameters, dietary composition, and methionine-protected amino acid supplementation. Statistical analyses were performed in R Studio, with meta-regression models incorporating study as a random effect. Backward stepwise selection was applied, and models were evaluated using root mean squared error (RMSE) and concordance correlation coefficient (CCC). The final models indicated that starch intake, forage percentage in total mixed ration (TMR), and dietary methionine content significantly influenced milk fat percentage. Additionally, interactions between specific amino acids (e.g., histidine) and fatty acids (e.g., palmitic acid) revealed synergistic and antagonistic effects on milk fat percentage, highlighting the complexity of nutrient interactions regulating milk fat production. Further research is warranted to refine these insights and develop targeted nutritional strategies that optimize milk fat yield in commercial dairy systems.

Key Words: amino acid, rumen protected methionine, milk fat

2507 The effect of replacing common protein sources with heat-treated soybean meal on whole-animal nitrogen utilization and excretion in lactating Jersey cows. K. A. Harpestad*¹, K. K. Buse¹, A. L. Carroll¹, P. H. Doane², and P. J. Kononoff¹, *¹University of Nebraska–Lincoln, Lincoln, NE, ²Ag Processing Inc., USA.*

The cow’s requirements for nitrogen (N) can be met by feeding different feedstuffs. The objective of this study was to evaluate the impact of feeding heat treated soybean meal (HTSBM; Aminoplus; Ag Process-

ing Inc., Omaha, NE) in partial or full replacement of 3 common high protein feedstuffs, namely soybean meal (SBM), canola meal (CM), and blood meal (BM) on whole animal N utilization. Four treatments containing the same proportions of forage but different concentrate mixes were made (DM basis); AP: a diet containing 10.3% HTSBM, 3.78% SBM, no CM, and no BM; BM+SBM: a diet containing no HTSBM, 9.60% SBM, no CM, and 2.22% BM, AP+CM: a diet containing 5.13% HTSBM, 5.00% SBM, 5.13% CM, and no BM, and CM+BM: a diet containing no HTSBM, 3.85% SBM, 8.05% CM, and 2.2% BM. These formulations all contained 17% CP and were fed to 12 multiparous Jersey cows (32.4 ± 11.7 MY and 104 ± 4 DIM) in a 4×4 Latin square with 4 periods of 28 d. Data were analyzed using PROC GLIMMIX in SAS (version 9.4, SAS Institute Inc.). The model included fixed effects of treatment, square, and period nested in square and the random effects of cow nested in square. Results indicate formulations containing HTSBM can replace major protein ingredients without major effects on whole animal N utilization. In fact, compared with CM+BM, urinary N excretion may be reduced and whole animal N utilization enhanced when HTSBM is fed with CM. Results of the experiment are shown in Table 1.

Key Words: nitrogen utilization, protein ingredients

2508 Effect of increasing lysine supply on milk and milk component yield. A. C. Benoit*¹, N. M. Fox¹, D. M. Barbano², I. Brown-Crowder³, V. Malacco³, E. Raffrenato³, and M. E. Van Amburgh¹, *¹Department of Animal Science, Cornell University, Ithaca, NY, ²Department of Food Science, Cornell University, Ithaca, NY, ³Kemin Industries, Inc., Des Moines, IA.*

The objective of this study was to evaluate the effect of increasing metabolizable Lys supply on DMI, milk, and milk components and fatty acids (FA). One hundred forty-four lactating cows (88 ± 28 DIM) were randomly allocated to 9 pens with 16 cows per pen in a 70-d longitudinal study. Following a 14-d covariate period, pens were stratified by ECM yield and assigned to: (1) a diet that supplied Lys at 2.81 g/Mcal ME, (2) a diet that supplied Lys at 2.98 g/Mcal ME, or (3) a diet that supplied Lys at 3.18 g/Mcal ME. A rumen-protected Lys prototype (Kemin Industries, Inc., Des Moines, IA) was supplemented to increase Lys supply by 0, 13, and 35 g/d for diets 1, 2, and 3, respectively. Diets were formulated with the Cornell Net Carbohydrate Protein System v6.5.5 and composed of 31% aNDFom, 24% starch, 6.2% sugar, 3.6% total FA, and Met and His supplied at 1.2g/Mcal ME. All treatments included

Table 1 (Abstr. 2507). Effects of feeding heat-treated soybean meal at different inclusion rates on fecal and urinary output and N excretion, secretion, and partitioning of lactating Jersey cows

Item	Treatment ^{1,2}				SEM	P-value
	AP	BM+SBM	AP+CM	CM+BM		
Mass, g/d						
N intake	614 ^b	699 ^{ab}	649 ^b	766 ^a	35.7	0.01
Fecal N	181 ^b	188 ^{ab}	188 ^{ab}	199 ^a	7.6	0.05
Urinary N	172 ^{ab}	176 ^{ab}	158 ^b	196 ^a	11.0	0.04
Milk N	153	158	159	158	6.6	0.72
Retained N	106	175	142	213	31.7	0.11
N efficiency						
Milk N/N intake	25.1 ^{ab}	25.2 ^{ab}	27.5 ^a	22.1 ^b	1.20	0.03

¹AP = Aminoplus diet, BM+SBM = blood meal and soybean meal diet, AP+CM = Aminoplus and canola meal diet, CM+BM = canola meal and blood meal diet.

²Least squares means; largest standard error of treatment mean is shown.

chromium propionate (Kemin Industries, Inc., Des Moines, IA). The statistical model included the fixed effect of treatment, week, and their interaction, the random effect of pen within treatment, and the covariate adjustment of cow within pen. Contrasts were used to test the linear and quadratic effects of increasing Lys supply. Results are presented in order of Lys inclusion at 2.81, 2.98, and 3.18 g/Mcal ME, respectively. Increasing Lys supply did not affect DMI (28.2, 28.1, and 27.5 kg/d; linear $P = 0.11$) or the yields of milk (46.4, 46.6, and 46.7 kg/d; linear $P = 0.30$) or ECM (49.5, 49.8, and 49.8 kg/d; linear $P = 0.50$). Increasing Lys supply did not affect the contents of milk fat (4.05%, 4.03%, and 3.98%; linear $P = 0.14$) or lactose (4.75%, 4.75%, and 4.76%; linear $P = 0.73$) but quadratically affected protein (3.14%, 3.18%, and 3.14%; quadratic $P = 0.03$) and MUN (8.48, 8.29, and 8.86 mg/dL; quadratic $P = 0.01$). Increasing Lys supply did not affect the contents (g/100 g milk) of de novo FA (<16 carbons; 1.13%, 1.14%, and 1.11%; linear $P = 0.26$), mixed FA (16-carbon; 1.52%, 1.53%, and 1.50%; linear $P = 0.13$), or preformed FA (>16 carbons; 1.17%, 1.14%, and 1.15%; linear $P = 0.35$). Understanding the requirement for Lys in the mammary gland is important for improving milk fat and protein synthesis.

Key Words: lysine, milk fat, amino acid

2509 Optimizing milk composition and yield with 2-hydroxy-4-methylthio butanoate isopropyl ester (HMBi) supplementation: Insights from a meta-analysis. N. L. Whitehouse^{*1} and L. Bahloul², ¹University of New Hampshire, Durham, NH, ²Centre of Expertise and Research in Nutrition, Adisseo, Commeny, France.

The objective of this study was to establish response equations for milk protein and fat contents and yields (MPC, MFC, MPY, and MFY) in response to supplementation with HMBi (2-hydroxy-4-methylthio butanoate isopropyl ester, MetaSmart), an effective source of methionine for ruminants, and to identify factors influencing these responses. A meta-analysis was conducted using peer-reviewed data from 16 treatments across 8 published studies. Lactating dairy cows, 85 ± 57 DIM, had an average dry matter intake (DMI) of 20.5 ± 3.91 kg/d, with diets containing 166.1 $\pm$ 7.81 g crude protein (CP)/kg DM, 2.65 $\pm$ 0.14 Mcal metabolizable energy (ME)/kg DM, and 0.98 $\pm$ 0.14 g metabolizable methionine (mMet)/Mcal ME. Robust within-study models were developed to predict MPC (adjusted $R^2 = 0.91$, RMSE = 0.06%), MPY (adjusted $R^2 = 0.80$, RMSE = 53.9 g/d), MFC (adjusted $R^2 = 0.88$, RMSE = 0.12%), and MFY (adjusted $R^2 = 0.90$, RMSE = 83.2 g/d) as a function of mMet supply (g/Mcal ME). The models indicated that increasing mMet by 0.29 g/Mcal ME resulted in increases of 0.16% and 82 g/d for MPC and MPY, respectively, with additional increases of 0.06% and 10.0 g/d during early lactation (0–60 DIM). Similarly, MFC and MFY increased by 0.20% and 107 g/d, respectively, with an additional rise of 0.39% and 126 g/d in early lactation. In conclusion, the responses of lactating dairy cows to HMBi supplementation can be accurately predicted. Ongoing research aims to identify interfering factors that may influence these responses. These findings provide deeper insights into the underlying biological mechanisms and could contribute to optimizing dietary formulations for improved dairy performance.

Key Words: meta-analysis, methionine, amino acid

2510 Comparison of rumen-protected methionine sources for lactating dairy cows. K. C. Krogstad^{*1}, I. G. Moussiaux¹, C. Lee¹, and J. de Souza², ¹Department of Animal Sciences, The Ohio State University, Wooster, OH, ²Perdue Agribusiness, Salisbury, MD.

Rumen-protected methionine (RPMET) is commonly fed to lactating dairy cattle because it increases milk yields, milk protein yields, and enhances immune function. There are multiple RPMET products commercially available, but they differ in how they are protected from rumen degradation. Our objective was to evaluate 2 differently protected RPMET products in diets fed to lactating dairy cows. We enrolled 22 Holsteins and 10 Jerseys into a crossover experiment with 28-d periods. The diets were identical except for the RPMET source (RPMET1, RPMET2). Both had pH sensitive polymer protection, but the materials of the polymer differed. Milk yield and feed intake were measured daily, body weight was measured thrice weekly, and body condition score was observed weekly. Milk component samples were collected twice per week at both milkings. Plasma samples were collected 4 h post-feeding on the final 2 d of each period. Data were analyzed with a mixed model that included the fixed effects of treatment, breed, and their interaction. The random effect was period and cow. Significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$. The treatment $\times$ breed interaction was significant ($P < 0.01$) such that Jerseys fed RPMET2 consumed 1.1 ± 0.55 kg/d (difference $\pm$ SED) less dry matter than cows fed RPMET1 ($P = 0.05$). Milk yield, yield of milk components, energy-corrected milk yield, and feed efficiency were not affected by RPMET ($P \geq 0.48$). Milk urea nitrogen increased for RPMET2 compared with RPMET1 (14.6 ± 0.40 mg/dL vs. 15.1 ± 0.40 mg/dL [Mean $\pm$ SEM]; $P = 0.02$). Milk protein concentration was 0.03 ± 0.013 percentage units (difference $\pm$ SED) greater for RPMET2 than RPMET1 ($P = 0.05$). Body condition score and BW change were not affected by treatment ($P \geq 0.18$). Plasma methionine concentration was greater for RPMET2 than RPMET1 (37.7 ± 1.06 vs. 32.7 ± 1.06 ; $P < 0.01$) while other plasma amino acids were similar for each RPMET ($P \geq 0.25$). In conclusion milk and component yields were similar between methionine sources but RPMET2 resulted in greater milk protein concentration, MUN, and plasma Met, which suggests there are nuanced differences probably associated with passage rate.

Key Words: amino acid, protein

2511 Effects of methionine analog on plasma amino acid and metabolism during a heat-stress challenge in post-peak lactating Holstein cows. E. S. O'Meara^{*1}, H. A. Tucker², and F. C. Cardoso¹, ¹University of Illinois, Department of Animal Sciences, Urbana, IL, ²Novus International, Inc., Chesterfield, MO.

This study aimed to evaluate the effects of methionine analog (METa; HMTBa [supplied as MFP feed supplement], Novus International, Inc., Chesterfield, MO), fed at 0.24% of DMI, to provide a sufficient post-ruminal methionine supply on plasma amino acid and metabolism in post-peak lactating, multiparous Holstein cows during a heat stress challenge (HSC). Thirty-two multiparous, lactating Holstein cows (balanced by DIM [169 ± 66]; body surface area [6.73 ± 3.2 m²]) were randomly assigned to 1 of 2 dietary treatments: TMR with METa or TMR without (CON). Within each dietary treatment group cows were randomly assigned to 1 of 2 environmental treatment groups in a split-plot crossover design. The study was divided into 2 periods with 2 phases each. In phase 1 (9 d), all cows were in thermoneutral conditions (TN) and fed ad libitum. In phase 2 (9 d), group 1 ($n = 16$) was exposed to a HSC using electric heat blankets. Group 2 ($n = 16$) remained in TN but was pair-fed (PFTN) to HSC counterparts. After a 14-d washout period, the study was repeated (period 2) and environmental treatments inverted relative to treatments from phase 2 of period 1, while dietary treatments (METa or CON) remained the same for each cow. Serum and

plasma samples were taken on d 1 and 9 of each phase 1 h after feeding. Statistical analyses were performed using the MIXED procedure of SAS. Cows in HSC had less serum bicarbonate (26.9 mmol/L) and sodium (138.8 mmol/L) than PFTN (27.6 and 139.1 mmol/L; respectively; $P < 0.05$). Cows in PFTN had greater serum alkaline phosphatase (46.0 U/L) and γ -glutamyl transferase (GGT; 30.9 U/L) than HSC (42.1 and 29.8 U/L; respectively; $P < 0.01$). Cows in METa had lower serum albumin (3.69 g/dL) and greater GGT (31.6 g/dL) than CON (3.82 g/dL and 29.1 U/L; respectively; $P < 0.04$). Cows in METa had greater plasma dispensable AA than HSC (115 and 109 μ M; respectively; $P = 0.04$). Cows in HSC had greater plasma branched-chain AA (70.3 and 67.3 μ M; respectively; $P = 0.05$). Cows in METa tended to have greater plasma Met concentrations than CON (3.20 and 3.00 μ M; respectively; $P = 0.11$). In conclusion, cows that were pair-fed may have experienced greater inflammation than cows exposed to heat stress. The inclusion of HMTBa at 0.24% of DMI provided greater Met and dispensable AA during an HSC.

Key Words: 2-hydroxy-4-(methylthio)butanoic acid (HMTBa), heat stress, plasma AA

2512 Effects of replacing porcine blood meal with rumen-protected lysine on performance and N use efficiency in high-producing dairy cows. U. Arshad^{*1}, S. J. Johnson¹, R. Fukumori¹, A. J. Bowers¹, S. J. Kendall¹, I. Brown-Crowder², V. Malacco², F. Sun², and H. M. White¹, ¹University of Wisconsin–Madison, Madison, WI, ²Kemin Industries, Inc., Des Moines, IA.

The objectives of this study were to determine the effects of replacing porcine blood meal (PBM) with rumen-protected Lys (RPL; LysiGEM Extend, Kemin Industries, Inc.) on lactation performance and N use efficiency (NUE) in dairy cows. Holstein cows ($n = 64$) with a mean $\pm$ SD lactation of 3.3 ± 1.0 , milk yield 51.5 ± 6.2 kg/d, and milk protein $3.38\% \pm 0.47\%$ were enrolled at 87 ± 18 DIM. Cows and gate feeders (RIC system, Insentec, Marknesse, the Netherlands; 16 cows/8 bins/treatment) were randomly assigned to one of 4 treatments for 12 weeks: (1) control diet with PBM as the sole source of metabolizable Lys (PBM); (2) 33% of the dietary metabolizable Lys from PBM replaced with RPL (RPL33); (3) 66% of the dietary metabolizable Lys from PBM replaced with RPL (RPL66); or (4) 100% of the dietary metabolizable Lys from PBM replaced with RPL (RPL100). The supply of metabolizable Met was similar between treatments without additional AA supplementation. Due to the replacement of PBM with RPL, RPL33, RPL66, and RPL100 had 27, 70, and 114 g/d less MP than required. Lactational performance and DMI were collected daily, and fecal, urine, and blood samples were collected during the covariate and 12th wks of the study. Data were analyzed using SAS, with fixed effects of RPL, time, and the interaction, and random effect of cow nested within treatment. Preplanned contrasts were used to determine linear, quadratic, or cubic effects. Replacing PBM with RPL did not affect BCS, BW, energy balance, N balance, or CH₄ yield. Replacing PBM with RPL tended to reduce DMI linearly ($P = 0.10$), while milk (49.6 vs. 47.6 vs. 47.8 vs. 45.9 $\pm$ 0.50 kg/d) and protein yields (1.55 vs. 1.49 vs. 1.48 vs. 1.45 $\pm$ 0.02 kg/d) and MUN were linearly reduced ($P < 0.0001$). Intake of N was linearly ($P = 0.006$) reduced, and NUE maintained, with increasing replacement of PBM with RPL. Concentrations of BUN tended ($P = 0.09$) to decrease linearly with increasing replacement of PBM with RPL. Yield of ECM (50.8 vs. 49.6 vs. 49.6 vs. 48.8 $\pm$ 0.71 kg/d) did not differ among PBM, RPL33, and RPL66; however, feeding RPL100 tended to reduce ECM yield ($P = 0.08$). Overall, partial replacement of PBM with RPL is feasible without affecting ECM or NUE. Fully replacing PBM with RPL without compensating maintenance of MP

supply resulted in decreased ECM yield, highlighting the importance of adequate MP supply while optimizing individual AA.

Key Words: N balance, production, rumen-protected lysine

2513 Effects of metabolizable protein supply on metabolic function of CD4⁺ T cells in dairy cows. U. Arshad^{*1}, T. Fernandez-Wallace¹, R. Fukumori¹, S. J. Johnson¹, S. J. Kendall¹, L. R. Cangiano¹, I. Brown-Crowder², V. Malacco², F. Sun², and H. M. Heather¹, ¹University of Wisconsin–Madison, Madison, WI, ²Kemin Industries Inc., Des Moines, IA.

The objective was to determine the effects of MP supply on CD4⁺ T cells metabolic function in dairy cows. Holstein cows ($n = 32$) with a mean $\pm$ SD lactation of 3.28 ± 0.99 , milk yield 51.6 ± 6.1 kg/d, and milk protein $3.38\% \pm 0.47\%$ were enrolled at 86 ± 18 DIM in a pen with 16 gate feeders. Cows and gate feeders (RIC system, Insentec, Marknesse, the Netherlands; 16 cows/8 bins/treatment) were randomly assigned to one of 2 treatments for 12 wk: (1) diet adequate in MP supply (MPA, MP = 3,111 g/d) or (2) diet deficient in MP supply (MPD, MP = 2,983 g/d) by replacing porcine blood meal with rumen-protected Lys (LysiGEM Extend, Kemin Industries, Inc.), with similar metabolizable Met and without additional AA supplementation. Blood was collected (wk 11) to isolate peripheral blood mononuclear cells followed by magnetic isolation of CD4⁺ T cells. The isolated CD4⁺ T cells from each cow were split into 2 tubes and randomly assigned to incubate in an assay medium or with phorbol myristate acetate and ionomycin to evaluate metabolic function under metabolically nonactivated (NAC) and activated (AC) states, respectively, using an extracellular flux analyzer. Mitochondrial and glycolytic functional kinetics were recorded and based on real-time measurement of oxygen consumption rate and extracellular acidification rate (ECAR) under basal conditions and in response to complex V inhibitor (oligomycin), a protonophore uncoupler (BAM 15), and complex I and III inhibitors (rotenone and antimycin A, respectively). A split-plot design analysis in SAS was employed to account for diet, activation state, responsiveness over assay time, and their interactions, along with random effects and errors. The maximal respiration rate (MRR) and sparing respiratory capacity (SRC) in cows fed MPD were reduced ($P \leq 0.006$) in both NAC (MPA = 48.9 vs. MPD = 35.5 $\pm$ 5.8 pmol/min) and AC (MPA = 31.6 vs. MPD = 19.6 $\pm$ 4.6 pmol/min) CD4⁺ T cells compared with MPA. A diet $\times$ time interaction ($P = 0.01$) for ECAR indicated that MPD-fed cows showed decreased aerobic glycolysis over time within the assay. The decrease in ECAR, along with reduced MRR and SRC, indicates a decline in metabolic fitness in cows fed MPD diets, which may impact immune function during periods of nutrient imbalance.

Key Words: amino acid, seahorse, T cell

2514 Rumen-protected aromatic amino acid supplementation increases plasma amino acids and hepatic fatty acid oxidation in dry dairy cows. G. Ahmad^{*}, J. R. Daddam, M. Sura, and Z. Zhou, Michigan State University, East Lansing, MI.

Aromatic amino acids (AAA) serve as protein building blocks and key precursors for bioactive molecules that play essential roles in regulating various biological functions. This study aimed to evaluate the effects of rumen-protected aromatic amino acid (RP-AAA) supplementation on plasma amino acid (AA) profiles and liver fatty acid oxidation in dry cows. Ten nonlactating pregnant multiparous Holstein cows were enrolled in a complete randomized design right after drying off. Cows were top-dressed for the first 7 d post-drying-off with (1) without

RP-AAA (CON, $n = 5$); or (2) RP-AAA (61 g tryptophan, 117 g phenylalanine, and 102 g tyrosine, $n = 5$). All cows received the same far-off diet containing 13.83% CP on a dry matter basis. Treatment effects were determined using PROC GLIMMIX in SAS. Blood samples were collected at 0, 3 and 7 d relative to supplementation for quantification of 20 proteinogenic amino acids with LC-MS. Liver tissue was harvested at 0 and 7 d relative to supplementation for ^{14}C -palmitate oxidation assays. Plasma AAA concentrations were greater in RP-AAA cows compared with CON ($P = 0.02$), indicating successful rumen protection and absorption of the supplement. Dry matter intake tended to be higher in cows receiving RP-AAA 13.96 kg/d compared with CON 11.14 kg/d ($P = 0.09$). Rumen-protected AAA supplementation increased plasma levels of total AA ($P = 0.01$), essential AA ($P = 0.01$), branched-chain AA ($P = 0.02$), and total nonessential AA ($P = 0.05$) compared with CON. Among individual AAs, tryptophan and tyrosine ($P = 0.03$) were greater in RP-AAA cows, while concentrations of plasma phenylalanine were not changed ($P > 0.10$), likely due to its conversion into tyrosine in the gut and liver. Similarly, arginine ($P = 0.02$), methionine ($P = 0.03$), glycine ($P = 0.04$), and asparagine ($P = 0.01$) were also greater in RP-AAA cows compared with CON, while lysine ($P = 0.07$) and threonine ($P = 0.09$) tended to be greater in RP-AAA cows compared with CON. Cows receiving RP-AAA also tended toward higher oxidation of palmitate to CO_2 compared with CON ($P = 0.07$), suggesting enhanced fatty acid oxidation in the liver. Overall, the increased availability of proteinogenic AAs suggests a potential beneficial role for RP-AAA supplementation. Further research is needed to elucidate the specific changes in AA metabolism and their impact on postpartum lactation performance.

Key Words: dairy cow, dry period, aromatic amino acid

2515 Evaluation of branched-chain volatile fatty acids and nitrate on lactation performance of dairy cows fed low-protein diets. P. Piantoni¹, K. Dieho², D. Tomlinson³, D. Kleinschmit³, and G. Schroeder¹, ¹Cargill Animal Nutrition and Health, Global Innovation Center, Elk River, MN, ²Cargill Animal Nutrition and Health, Global Innovation Center, Veldriel, the Netherlands, ³Zinpro Corporation, Eden Prairie, MN.

To evaluate the effect of branched-chain volatile fatty acids (BCVFA) and calcium ammonium nitrate (NIT) on lactation performance, 8 rumen-fistulated Holstein-Friesian cows (44.3 ± 8 kg/d milk yield; 119 ± 62 DIM; mean $\pm$ SD) were used in a 4×4 Latin square design experiment with 28-d periods and a 2×2 factorial arrangement of treatments. Factors were inclusion of NIT (NIT⁻ or NIT⁺, with 0% or 1% of diet DM as nitrate) and BCVFA (BCVFA⁻ or BCVFA⁺, with 0 or 40 g/d as is of BCVFA). Diets (NIT⁻ and NIT⁺) were isonitrogenous and 58.8% forage, 33.1% NDF, 24.4% total starch, and 13.5% CP. Supplemental NIT replaced dietary true protein sources in NIT⁺. Data from the last week of each period were analyzed using the Lme4 package in R with a model containing the fixed effects of BCVFA, NIT, and their interaction, and random effects of square, period, and cow. Treatments did not affect milk yield (38.9 ± 2.62). However, BCVFA⁺ decreased ($P_{\text{NIT} \times \text{BCVFA}} = 0.08$) milk fat yield by 160 g/d and tended ($P_{\text{NIT} \times \text{BCVFA}} = 0.14$) to decrease 3.5% FCM yield by 3.2 kg/d only when fed in NIT⁻. In addition, BCVFA⁺ decreased ($P_{\text{NIT} \times \text{BCVFA}} = 0.09$) DMI in NIT⁺ (25.5 vs. 26.6 kg/d), increasing feed efficiency ($P_{\text{NIT} \times \text{BCVFA}} = 0.01$), with opposite

effects in NIT⁻. Overall, NIT⁺ did not affect DMI or feed efficiency, but decreased milk CP yield (1.24 vs. 1.31 kg/d) and content (3.24% vs. 3.38%; both $P < 0.05$) and tended to decrease milk urea (12.6 vs. 13.6 mg/dL; $P = 0.09$). Moreover, NIT⁺ decreased NDF digestibility (58.3% vs. 60.0%) and time below rumen pH 5.8 (5.0 vs. 8.5 h; both $P < 0.05$). No effect of BCVFA was detected for NDF digestibility. Overall, BCVFA⁺ increased rumen isobutyrate concentration (1.03 vs. 0.84 mM; $P < 0.01$) but not total VFA concentration, while NIT⁺ increased rumen ammonia (6.56 vs. 5.53 mg/dL) and decreased total VFA concentration (148 vs. 153 mM) compared with NIT⁻ (both $P < 0.05$). Supplementation of BCVFA increased feed efficiency in NIT⁺ but decreased it in NIT⁻ with limited effects on rumen parameters evaluated. Overall, replacing true protein sources with NIT decreased production performance in cows fed low-CP diets.

Key Words: lactating cows, low-protein diet, nitrate

2525 Cow-level evaluation of plasma essential amino acid clusters and their association with lactating dairy cow performance. G. L. Menezes¹, P. Letelier¹, J. R. R. Dorea¹, M. L. Sullivan², L. A. Reinhardt², G. I. Zanton², and M. A. Wattiaux¹, ¹University of Wisconsin-Madison, Madison, WI, ²United States Department of Agriculture-Agricultural Research Service, Madison, WI.

Studying changes in the profile of plasma EAA (pEAA) may provide a foundation for precision protein nutrition strategies for dairy cows. A previous systematic review identified 2 pEAA clusters that were associated with cow performance, but controlled trials are necessary to evaluate these findings on individual cow-level data. This study aimed to validate the clusters identified in the review using a supervised machine learning model in controlled research setting and evaluate dairy cow performance across different pEAA clusters. A Random Forest algorithm was trained for cluster identification using data from the systematic review, with Arg, His, Ile, Leu, Lys, Met, Phe, Thr and Val as inputs and clusters as outputs. A leave-one-trial-out cross-validation approach evaluated the model's performance, achieving accuracy, precision, recall, and F1-score of 84.4%, 87.3%, 85.7%, and 86.5%, respectively. The model was then retrained using the entire dataset to predict clusters for 62 cows across 4 lactation stages receiving diets with CP levels of 13.6%, 15.2%, 16.7%, and 18.3% over an 8-wk trial. The pEAA profile was assessed in wk 4 and 8, where 33 and 38 cows were classified as cluster A, and 29 and 24 as cluster B, respectively. Cows in cluster B exhibited higher concentrations of Arg (12%), His (26%), Ile (15%), Leu (34%), Lys (17%), Met (12%), Phe (16%), Trp (4%), and Val (26%) and produced 2.3% more ($P = 0.047$) fat- and protein-corrected milk (39.8 ± 0.49 vs. 38.9 ± 0.46 kg/d). They also showed a 2.5% increase ($P = 0.001$) in DMI (28.4 ± 0.23 vs. 27.7 ± 0.22 kg/d), a 3.0% increase ($P = 0.008$) in lactose yield ($1,874 \pm 24$ vs. $1,820 \pm 23$ g/d), and a 2.8% decrease ($P = 0.033$) in NUE (28.5 ± 0.42 vs. 27.7 ± 0.44 %). Despite increased milk production, the pEAA profile was not associated with milk composition or feed efficiency. These findings validate the group-level association observed between the AA profile and dairy cow performance at the individual cow level.

Key Words: plasma amino acid profile, nutrient use efficiency, cluster analysis

Teaching Undergraduate and Graduate Education 1: Posters

2516 Research project incorporation in a dairy production course. B. W. Jones*, *Eastern Kentucky University, Richmond, KY.*

The objective of this study was to evaluate students' research project experience, concepts acquired, classroom knowledge, and soft skills gained from participating in an in-class research project. During the fall 2024 semester, an in-class research project was incorporated in the dairy production course at Eastern Kentucky University. The class partnered with the Eastern Kentucky University Meadowbrook farm and charged the students with conducting a project during class. Throughout the semester students focused on the impact of behavior in calves dehorned with or without pain management. Students ($n = 11$) worked in 2 groups as the project emphasized collaborative learning. Following completion of the project, a paper survey was distributed to the students. Completion of the anonymous survey was voluntary. The overall response rate was 100%. The survey consisted of 17 questions over classroom knowledge, research project concepts, and research project experience. Fifteen of the questions were quantitative and 2 were

qualitative. Statistical analysis was conducted on survey responses using SAS (Version 9.4, SAS Institute Inc., Cary, NC). The FREQ procedure was used to determine percentage of responses. Overall, 100% of the students recommend that this course continue to work with the farm to offer research projects during course time and 63% of students identified their specific project experience as positive. Eighty-one percent of the students agreed or strongly agreed that participating in the project increased their understanding of the importance of pain management during dehorning. Student self-identified soft skills enhanced in the project were leadership, teamwork, time management, collaboration, communication, and research skills. Implementing a research project into the dairy curriculum was useful for the students as it increased their knowledge of the importance of pain management during dehorning and enhanced their soft skills. Plans continue to implement research projects into each semester the dairy production course is taught.

Key Words: teaching, student research, experiential learning

Animal Behavior and Well-Being 2: Posters

2600 Association between the time budget of primiparous cows and their adaptation to an automatic milking system following parturition. L. M. Stewart, J. E. Brasier*, and T. J. DeVries, *Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

The study objective was to identify the differences in the transition period time budget of first lactation cows who adapt quickly to a free-traffic automatic milking system (AMS) versus those who require more chronic fetching during adaptation following calving. Twenty-two primiparous Holstein cows were observed from 21 d before calving to 23 DIM, following their adaptation to an AMS. Prepartum and postpartum cow time budgets were monitored automatically for feeding, rumination, and lying behavior, and also monitored for milking metrics and fetching behavior postpartum. Cows were designated as either high fetch (HF; $n = 11$) or low fetch (LF; $n = 11$) based on their average fetch frequency from 8 to 23 DIM, with HF averaging ≥ 0.5 fetches/d and LF < 0.5 fetches/d. Differences in cow time budgets and production were analyzed with mixed-effect, repeated measures linear regression models. High-fetch cows tended to eat slower in both the prepartum (0.06 vs. 0.08 kg DM/min; SE = 0.008; $P = 0.08$) and postpartum periods (0.10 vs. 0.12 kg DM/min; SE = 0.01; $P = 0.08$) and spend more time at the feed bins both prepartum (223.2 vs. 195.2 min/d; SE = 15.0; $P = 0.08$) and postpartum (183.4 vs. 155.3 min/d; SE = 12.0; $P = 0.03$) compared with LF cows. Lying duration was similar ($P > 0.5$) both prepartum (818.7 min/d) and postpartum (587.4 min/d). Across the transition period HF cows tended to ruminate for less time than LF cows (prepartum = 485.9 vs. 505.7 min/d; SE = 11.2; $P = 0.09$; postpartum = 484.9 vs. 517.4 min/d; SE = 16.9; $P = 0.07$), despite similar ($P \geq 0.4$) total DMI (prepartum = 12.0 kg/d DM; postpartum = 16.6 kg/d DM). The HF cows had fewer milkings (2.9 vs. 3.8 milkings/d; SE = 0.11; $P < 0.01$) and lesser milk yield (26.6 vs. 29.4 kg/d; SE = 0.44; $P < 0.01$) than LF cows. The HF cows also spent more time in the fetch pen than LF cows (16.9 vs. 5.2 min/d; SE = 3.3; $P < 0.01$). The results indicate there may be features of a cow's prepartum time budget that relate to their subsequent ease of adaptation to the AMS, and that in the postpartum period both HF and LF cows adapted their time budgets to account for added time in the fetch pen and additional visits to the AMS, respectively.

Key Words: activity budget, robotic milking, adaptation

2601 Effect of personality traits of primiparous dairy cows on their adaptation and response to prepartum training to an automated milking system. J. E. Brasier*, D. B. Haley², R. Bergeron¹, and T. J. DeVries¹, ¹*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* ²*Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

The objective of this study was to determine how the personality of a cow affects their adaptation to an automated milking system (AMS), and their response to being trained or not on the AMS prepartum. Thirty pregnant Holstein heifers were enrolled 21 d before their expected calving date and assigned to 1 of 2 treatments: control (CON; no training; $n = 16$), and training (TRN; included exposure to AMS environment, concentrate, and mechanical arm and noises; $n = 12$). The TRN cows were trained on the AMS 14 d before calving for 3×/d for 4 d. All cows calved in singular maternity pens and were then moved to the AMS pen to be milked by the AMS. Ease of entry to the AMS (0 = unassisted to 6 = heavy assistance) was recorded for the first 6 milkings. Milking behavior and time spent in the fetch pen (min/d) were recorded for the

first 21 d. Cows were assessed for personality after the 21-d period using a combined arena test consisting of 3 parts: a novel arena test, a novel object test, and a novel human test. Behavioral observations from personality testing were analyzed using principal component analysis (PCA) to determine personality traits and associated PCA factor scores for each cow for the identified personality traits. The PCA revealed a factor interpreted as the personality trait of bold-vocal (factor score range: -1.3 to 2.5). The effect of personality trait (factor score) and treatment were analyzed using mixed-effect linear regression models. During training, TRN cows that scored high for bold-vocal had a worse ease of entry score on training d 3 (ease of entry score = $0.78 \times$ bold-vocal score + 3.8 ; $P = 0.04$) and 4 (ease of entry score = $0.92 \times$ bold-vocal score + 3.8 ; $P = 0.03$) compared with less bold-vocal TRN cows. There were treatment $\times$ factor score interactions ($P \leq 0.05$) for milking outcomes. Specifically, TRN cows had no association with factor score, but for CON cows, greater factor scores were associated with more fetching events (fetches[#/d] = $0.18 \times$ bold-vocal score + 0.95 ; $P < 0.01$) and more time spent in the fetch pen (time[min/d] = $1.91 \times$ bold-vocal score + 13.6 ; $P = 0.01$). Overall, the results demonstrate that primiparous cows of certain personality types may require more training on the AMS to improve adaptation.

Key Words: training, personality, robotic milking

2602 Patterns of milking behavior of dairy cows in automated milking systems. W. R. Silva^{1,2}, C. J. McWilliams^{1,3}, M. N. Pereira², and T. J. DeVries*, ¹*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* ²*Universidade Federal de Lavras, Lavras, MG, Brazil,* ³*Grand Valley Fortifiers, Cambridge, ON, Canada.*

The objective was to evaluate the milking behavior within a day of dairy cows in automated milking systems (AMS) across seasons and stages of lactation. A total of 447 (175 primiparous [PP] and 272 multiparous [MP]) cows with 0 to 150 DIM from 4 Canadian dairy farms were evaluated from May 2022 to April 2023. All farms used AMS (3 to 11 AMS units/farm) set up for free-traffic in freestall barns. The variables of interest were: milk yield per visit, box time, and milking interval. Outliers (mean $\pm 3 \times$ SD) were removed from the dataset. A total of 501,413 milking visits were evaluated. The data were grouped by season (fall, winter, spring, and summer) before analysis. Days in milk were classified into 5 groups (0–30, 31–60, 61–90, 91–120, and 121–150 DIM), and the day was split into 24 hourly intervals. Data were analyzed as repeated-measures with mixed models that included the fixed effects of farm (1–4), parity (PP or MP), DIM group (1–5), hour of the day (1–24), and 2- and 3-way interactions of parity, DIM, and hour. Cow within farm was added as random effect. Milking frequency averaged 3.3 ± 0.13 visits/d for PP and 3.5 ± 0.10 visits/d for MP cows. There was a 3-way interaction for all variables ($P < 0.01$). Primiparous cows had lower milk yield per visit than MP in all seasons (11.3 vs. 14.2 kg/visit; $P < 0.01$), but box time was longer for PP than MP in spring (7.1 vs. 6.8 min/visit; $P < 0.01$). Milking interval was longer for PP than MP in all seasons (7.6 vs. 7.3 h; $P < 0.01$). Milk yield per visit was the lowest for cows at 0–30 DIM in all seasons (11.2 kg/visit; $P \leq 0.05$), and the highest for cows at 61–90 DIM in fall, spring, and summer (13.4 kg/visit; $P \leq 0.05$). Box time was longer ($P \leq 0.05$) for cows at 31–60 DIM (7.1 min/visit), followed by 61–90, 91–120, 121–150, and 0–30 DIM (7.0, 6.9, 6.8, and 6.6 min/visit, respectively). As compared with the first 120 DIM, cows at 121–150 DIM had a longer milking interval

in the fall, winter, and summer (7.9 vs. 7.4 h; $P \leq 0.05$), but not in spring ($P > 0.05$). The maximum milk yield per visit occurred between 0600 and 1000 h (13.9 ± 1.98 kg/visit), and the minimum between 2000 and 0000 h (12.0 ± 1.51 kg/visit). Results demonstrate that AMS milking behavior varies considerably with parity, DIM, time of day, and season.

Key Words: robotic milking, box time, milking interval

2603 Exploration of premilking behaviors: Order of entrance to holding pen and of start of milking. P. Bacigalupo Sanguesa^{*1,2}, S. Manski¹, P. L. Ruegg¹, R. Thomson¹, B. Norby¹, and R. J. Erskine¹, ¹Michigan State University, East Lansing, MI, ²Michigan State University Extension, East Lansing, MI.

With the intent of evaluating premilking behavioral consistency, we investigated the hierarchical order of entrance to the holding pen and the time to the start of milking. We enrolled commercial farms milking 3 times a day and equipped with milking parlor recording software. We randomly selected 35 cows from 6 different groups across 3 farm ($n = 210$) and followed them for 13 consecutive milkings. For visual identification, cows were marked with a unique colored symbol on both rump areas. We placed time lapse cameras at the entrance of the holding pens to determine time of entrance (TE) and used the parlor recording software to obtain time of start of milking (SM) for every milking. For each group, the TE and SM ranks of the milkings of each cow were averaged over the 13 milkings. After removing unusable data, our sample had 168 cows. To test if the order of entrance to the holding pen and to start of milking (i.e., TE rank and SM rank) were random, we compared the cows' average TE and SM rank to the distribution of average ranks that would be expected if the order of cows was random. We obtained individual P -values. Next, we calculated (overall and for each group) the proportion of cows for which these P -values were significant at the 0.05 level. For true randomness, a proportion of 0.05 or less is expected. The overall proportion of average TE ranks that were significant was 0.369 and the overall proportion of average SM ranks that were significant was 0.452. Table 1 shows the proportions by group. We conclude that while many cows behave "randomly" regarding their order of entrance to the holding pen and to the start of milking, a large proportion of them are consistent in their behavior such that their average ranks were outside of the range expected under true randomness. This might suggest that some cows have a preference in their order to arrive at the holding pen and when to start milkings.

Key Words: premilking, behavior

2604 Effect of music as a stimulus on milk production and quality in Holstein cows under tropical conditions. J. R. Romero², L. F. Osorio¹, R. Espinal¹, and M. Moncada-Lainez^{*1}, ¹Escuela Agrícola Panamericana Zamorano, Tegucigalpa, Honduras, ²Smithfield Foods, USA.

Table 1 (Abstr. 2603). Proportion of cows with average TE ranks and SM ranks with $P < 0.05$ by group

Group	Average TE rank	Average SM rank
A	0.5	0.54
B	0.38	0.58
C	0.4	0.47
D	0.13	0.43
E	0.37	0.33
F	0.46	0.39

The global demand for milk is increasing at a faster rate than production, highlighting the need for methods to improve both yield and quality. This study, conducted at the Panamerican Agricultural School, Zamorano, Honduras, aimed to assess the effect of music exposure at a volume of no more than 80 dB on milk production and its physicochemical properties in multiparous Holstein cows. To conduct the experiment, two 1,500-W speakers were installed in the milking parlor and an experimental paddock. The music played during the study was carefully selected from a Spotify playlist, with each song manually reviewed to ensure it met the criteria of ≤ 100 bpm. The cows were exposed to the selected music for half a day, from 5:00 a.m. to 5:00 p.m., in both the waiting area before milking and during milking, as well as in the paddock. The experimental design followed a randomized complete block design (RCBD) with 20 multiparous Holstein cows divided into 2 groups. The cows were milked twice a day. One group was exposed to music for 12 h each day, while the other group served as the control. After 3 wk, the treatments were switched, with a 1-wk adaptation period between treatments. Statistical analysis was performed using ANOVA with Statistical Analysis Software (SAS), and significant differences were determined using Duncan's test. The study measured milk yield, fat content, protein content, acidity, and freezing point. Results showed that music exposure led to a 5%–7% increase in milk production ($P \leq 0.05$) with no significant changes observed in fat content, crude protein, freezing point, or acidity of the milk between the 2 groups ($P > 0.05$). In conclusion, exposure to music with the specified criteria resulted in a 5%–7% increase in milk production without compromising the milk's quality. This suggests that music could be a cost-effective method to enhance milk yield without altering milk composition.

Key Words: animal welfare, milk composition, milk yield

2605 Easing the transition: Gradual versus abrupt dry-off in an automatic milking system. E. C. McGuire^{*1}, R. Pralle², J. Van Os¹, K. Creutzinger³, J. Laporta¹, J. R. R. Dorea¹, and M. E. Montes¹, ¹Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI, ²Department of Animal and Veterinary Sciences, University of Wisconsin–Platteville, Platteville, WI, ³Department of Animal, Dairy and Veterinary Sciences, University of Vermont, Burlington, VT.

Dairy cows are typically dried-off abruptly, which may negatively affect welfare due to udder engorgement after milking ceases. Gradually reducing milking frequency to taper milk production before milking cessation may lessen udder engorgement and discomfort, improving welfare. The objective of this ongoing study is to determine the effect of gradual vs. abrupt dry-off in an automatic milking system (AMS) on milk yield, udder engorgement, and pain. Holstein cows were enrolled 74 ± 3 d before expected calving (target $n = 18$ /treatment $n = 12$ /treatment completed). Gradually dried-off cows had a 2 wk step-down in milking frequency based on their 10-d average milking frequency before enrollment, while the abrupt group had no change in management until dry-off. Both groups were dried off 60 ± 3 d before expected calving. Milk yield (kg/d) was recorded until dry-off. Udder firmness (firm/very firm vs. normal/soft), response to palpation (leg raising, tail wagging, withdrawal or vocalizing), and milk leaking (≥ 1 teat) were assessed on d 1, 3, and 5 post dry-off. Difference (Δ) in daily milk yield from 14 to 1 d before dry-off was compared between treatments using a t -test. Cows in the gradual treatment had a greater reduction in milk yield than those in the abrupt treatment (mean $\pm$ SE: -12.7 ± 1.4 vs. -2.1 ± 1.2 kg/d; $P < 0.001$). Table 1 shows descriptive data on the number of cows displaying each udder characteristic. Our preliminary results suggest that cows experience less pain during palpation and have less udder engorgement when dried-off gradually compared with abruptly,

Table 1 (Abstr. 2605). Number of cows (n = 12/treatment) showing each udder characteristic when dried off abruptly (ADO) vs. gradually (GDO)

Udder characteristic	Days after dry-off					
	1		3		5	
	ADO	GDO	ADO	GDO	ADO	GDO
Firm or very firm udder	9	6	9	6	6	3
Behavioral response to udder palpation	2	4	5	3	4	0
Milk leakage present	8	3	8	1	4	2

likely due to lower milk yield at dry-off. Tailored gradual dry-off in an AMS may offer management to better meet individual cow needs.

Key Words: welfare, robotic milking, milking frequency

2606 Monitoring play behavior in dairy calves using computer vision and accelerometers. H. Yang^{*1}, E. Liu¹, J. Sun¹, D. Seeman¹, A. Jain¹, H. Lesscher², S. Steenbergen², C. Kamphuis², E. Visser², I. de Graaf², S. Vreuls², and M. Hostens^{1,3}, ¹*College of Agriculture and Life Sciences, Cornell University, Ithaca, NY*, ²*Department of Population Health Sciences, Division Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands*, ³*Department of Animal Sciences and Aquatic Ecology, Faculty of Bioscience Engineering, Ghent University, Ghent, East Flanders, Belgium*.

Monitoring play behavior in dairy calves provides crucial insights into their welfare and development. Traditional methods rely on labor-intensive monitoring, so we developed 2 scalable, automated pipelines that use accelerometer and video data collected from 6 European farms to detect the daily ratio of play behaviors in dairy calves to support related legislation for calves' welfare. In the accelerometer pipeline, data recorded at 100 Hz on 3 axes were segmented into overlapping 300-ms sequences. A bidirectional LSTM autoencoder was trained exclusively on nonplaying sequences to learn normal behavior. Sequences with reconstruction errors above the 95th percentile were flagged as play

events. This method processed 47 h of data in 4 min and achieved a ROC-AUC of 0.87. For the video pipeline, we used 63 videos collected from 2 farms. Each video spanned 33 min and was annotated with the labels indicating their behaviors within the timeframes. These video segments were decoded to frames using decord, resized to 224 by 224, and later standardized using ImageNet mean and SD. After that, we processed them in the VideoMAE model in batches of 16 frames (0.64 s) to meet the model requirements. These embeddings included both spatial and temporal information, enabling us to differentiate playing behaviors from nonplaying. A neural network classifier trained on these embeddings achieved over 97% accuracy on cross-validation and test sets; however, performance dropped when applied to data from a new farm, highlighting the need for a larger, more diverse training dataset. Our current study showed promising results in detecting play behavior in dairy calves in real-time but still needs further improvements to achieve better generalization. And the vision pipeline could be upgraded to enable individual behavior detection. Future work will enhance feature engineering and refine segmentation methods and tracking methods for individual calf detection. Single-frame feature extraction models like Clip and DINOv2 will also be deployed to enable single-shot detection. These pipelines promise to reduce manual annotation labor, detect play behavior in real-time, and advance our understanding of calf welfare.

Key Words: computer vision, transformers, VideoMAE

Animal Health 3: Posters

2607 Postpartum calcium dynamics and milk production differ in herds fed a negative DCAD diet or zeolite. A. C. R. Seely^{*1}, K. A. Meister², B. L. Keifer², M. M. McCarthy², and S. S. Bascom², ¹*University of New Hampshire, Durham, NH*, ²*Phibro Animal Health Corporation, Teaneck, NJ*.

With the aim of maintaining blood Ca status in the early postpartum period, negative DCAD rations (nDCAD) or those containing zeolite A (ZA), are commonly fed to dairy cows in the dry period. Our objective was to explore the difference in postparturient blood Ca dynamics and milk production between dairy cows fed nDCAD or ZA during the late dry period. We conducted an observational cohort study on multiparous Holsteins on 9 herds in Minnesota. During the late dry period, a nDCAD ration was fed to achieve a urine pH of 5.5 to 6.0 on 4 herds (n = 290 cows), and ZA was fed on 5 herds (n = 531 cows). Milk yield was recorded through 12 wk of lactation and venous blood samples were collected on a subset of cows (nDCAD: n = 36, ZA: n = 43) at 2 and 4 DIM to analyze serum total Ca (tCa). To explore blood Ca dynamics, cows were classified as dyscalcemic (DYS) if 4 DIM tCa ≤ 2.15 mmol/L (nDCAD: n = 16 [44%], ZA: n = 22 [51%]), or eucalcemic (EUC) if 4 DIM tCa > 2.15 mmol/L (nDCAD: n = 20 [56%], ZA: n = 21 [49%]). Linear mixed models were used to analyze the difference in serum tCa at 4 DIM and milk yield for the first 12 wk of lactation between nDCAD and ZA cows, including the random effect of farm. Mean serum tCa was lower at 2 than 4 DIM ($P < 0.001$) and was lower for nDCAD cows compared with ZA cows ($P = 0.001$; nDCAD: 2.04 [95% CI = $1.99, 2.09$] mmol/L, ZA: 2.16 [2.11, 2.21] mmol/L). Cows fed nDCAD produced more milk than ZA cows ($P < 0.001$; nDCAD: 50.3 [49.5, 51.1] kg/d, ZA: 43.0 [42.5, 43.6] kg/d). For nDCAD cows, milk yield was greater in EUC cows compared with DYS cows ($P = 0.006$; EUC: 53.4 [49.1, 57.8] kg/d, DYS: 49.6 [44.8, 54.3] kg/d). For ZA cows, milk yield was similar between EUC and DYS cows ($P = 0.45$; EUC: 45.0 [42.0, 48.0] kg/d, DYS: 42.7 [39.6, 45.8] kg/d). Our findings in this observational study suggest that ZA may improve blood Ca in early postpartum; however, this may influence the cow's natural ability to adjust to the demands of lactation, potentially impacting long-term milk yield, and further investigations are necessary.

Key Words: negative DCAD, zeolite A, dyscalcemia

2608 Associations of the time of peak plasma haptoglobin, peak haptoglobin concentration, and number of days with elevated haptoglobin in the first week postpartum with milk yield in clinically healthy Holstein cows. N. Taechachoekvivat^{*}, L. Avila-Granados, J. Grantz, A. Ueda, and R. Neves, *Purdue University, West Lafayette, IN*.

The objective of this study was to assess the associations of time of peak haptoglobin (Hp), peak Hp concentration, and number of days with elevated Hp in the first week of parturition with milk yield in clinically healthy cows. Blood samples were collected from 513 cows from 2 herds at 1, 3, 5, and 7 DIM, and 428 clinically healthy cows remained for the final analyses. Spearman correlation coefficients showed correlations between time of peak Hp, peak Hp concentration, and number of days with elevated Hp; thus, separate linear mixed models were used to assess the association of each variable with milk yield in the first 12 wk of lactation. Time of peak Hp was categorized as early (1 or 3 DIM) or late (5 or 7 DIM). Peak Hp concentration remained as a continuous variable. Number of days with elevated Hp was categorized as 0, 1, or ≥ 2 d, using thresholds from a previous study from our group (primiparous:

Hp > 0.204 , > 0.437 , and > 0.308 g/L at 1, 3, and 5 DIM, respectively; multiparous: Hp > 0.204 , > 0.328 , > 0.304 , and > 0.130 g/L at 1, 3, 5, and 7 DIM, respectively). In second-lactation cows, there was a time of peak Hp $\times$ week interaction, where early-peak cows produced more milk in wk 1 and 2 compared with late peak. In multiparous cows, a 0.1 g/L increase in peak Hp concentration resulted in a 0.7 ± 0.1 kg/d decrease in milk in wk 1 to 4 ($P < 0.0001$). Primiparous cows with ≥ 2 d of elevated Hp produced 3.1 ± 1.3 kg/d less than cows with 1 d of elevated Hp ($P = 0.05$). In third-lactation cows, there was an interaction between the number of days with elevated Hp $\times$ week, where cows with ≥ 2 d of elevated Hp produced less milk than cows with 0 and 1 d of elevated Hp, and cows with 1 d of elevated Hp produced less milk than cows with 0 d of elevated Hp. Peak Hp concentration and number of days with elevated Hp were negatively associated with milk yield, with more associations in multiparous cows. Time of peak Hp was only associated with milk yield in the first 2 wk in second-lactation cows.

Key Words: systemic inflammation, haptoglobin dynamics

2609 Association of colostrum yield and immunoglobulin concentration with dyscalcemia in multiparous Holsteins. K. R. K. Gottwald^{*} and J. A. A. McArt, *Department of Population Medicine and Diagnostic Science, Cornell University College of Veterinary Medicine, Ithaca, NY*.

During the transition period, cows experience metabolic disruptions, such as dyscalcemia, that impact immune function, health, and milk production. Metabolic stress in late gestation influences colostrum yield and quality, with cows in better energy balance producing more colostrum with higher IgG concentrations. Our objective was to compare colostrum characteristics between cows with normal versus low blood calcium concentrations at 4 DIM. Multiparous Holstein cows (n = 45) were enrolled from September to November on a research dairy where they were housed in tiestalls and milked thrice daily. Colostrum was harvested into a bucket within 8 h of calving, weighed, and a 1-L proportional sample collected for IgG analysis. Blood samples at 4 DIM were used for measurement of total calcium (tCa) from which cows were classified as "eucalcemic" (tCa ≥ 2.2 mmol/L, n = 33) or "dyscalcemic" (tCa < 2.2 mmol/L, n = 12). Multivariable linear regression models were built using stepwise backward selection. Colostrum yield ranged from 0.6 to 38.0 kg and IgG concentration from 66.8 to 205.1 g/L. Dyscalcemic cows produced 3.7 kg less colostrum than eucalcemic cows (dyscalcemic = 7.2 ± 1.9 kg, eucalcemic = 11.3 ± 1.1 kg; $P = 0.06$). Parity ≥ 4 cows produced 11.4 kg less colostrum than parity 2 (2.6 ± 2.0 kg vs. 14.0 ± 1.9 kg, respectively; $P < 0.01$) and 8.6 kg less colostrum than parity 3 (2.6 ± 2.0 kg vs. 11.1 ± 1.8 kg, respectively; $P < 0.01$). No difference was found in colostrum IgG concentration between dyscalcemic and eucalcemic cows (128.8 ± 8.3 g/L vs. 125.6 ± 4.7 g/L, respectively; $P = 0.70$); however, each kilogram increase in colostrum yield was associated with a 2.8 ± 0.6 g/L decrease in IgG concentration ($P < 0.01$). In summary, there was evidence of dyscalcemic cows having reduced colostrum yield compared with eucalcemic cows, but this was not associated with a change in IgG concentrations. Future work will explore associations of dyscalcemia with colostrum mineral concentrations and oxidant status index.

Key Words: dyscalcemia, colostrum production, colostrum immunoglobulin

Table 1 (Abstr. 2610). Least squares means for the relative frequencies of lameness diagnosis¹ by premilking waiting time (WT) category divided into quartiles (Q1 = shortest 25% WT), presented by parity category and genetic group

Parity and group	Waiting time quartile			
	Q1	Q2	Q3	Q4
Primiparous				
Holstein	0.1	0.033	0.12	0.055
Jersey	0.1	0.08	0.11	0.10
Holstein × Jersey	0.01	0.09	0.01	0.04
Multiparous				
Holstein	0.20	0.20	0.16	0.21
Jersey	0.25	0.22	0.22	0.30
Holstein × Jersey	0.21	0.17	0.27	0.25

¹No statistically significant differences in lameness diagnosis frequencies among quartiles were identified.

2610 Association of average premilking waiting time with lameness diagnosis in cows in an automated batch milking system. R. W. Zheng^{*1}, J. Velez², N. Rodriguez², and P. Pinedo¹, ¹Colorado State University, Fort Collins, CO, ²Aurora Organic Farms, Platteville, CO.

The objective was to test the association between average premilking waiting time (WT) and the diagnosis of lameness in cows managed in a semivoluntary batch milking system. The study used data from 614,951 milking events in 2,873 cows from February 2024 to September 2024 in a commercial organic grass-fed dairy in Texas, housing Holstein (HO), Jersey (JE), and Holstein × Jersey (HJ) cows. Cows were moved twice per day to the milking barn where they could select their milking visits among 22 automatic milking system units (DeLaval, Sweden). Visit information was collected to calculate premilking WT (min) in each milking event. Waiting time was defined as the time between the entrance of the group to the holding pen and the entrance of each individual cow to the robot milking box. Diagnosis of lameness was completed weekly by farm personnel. Average WT before lameness diagnosis in lame cows or for the whole lactation in unaffected cows were categorized into quartiles within parity (primiparous; multiparous) and genetic group (HO, JE, HJ). Data were examined separately in primiparous and multiparous cows. Least squares means for the relative frequency of lameness diagnosis by genetic group were calculated and compared using ANOVA. The relative frequencies of lameness diagnosis by WT quartile, parity category, and genetic group are presented in Table 1. Longer average WT was not associated with greater lameness frequency. Further research is needed to better understand the effect of premilking waiting time on lameness.

Key Words: lameness, waiting time

2611 Random forest algorithm predicting subclinical hyperketonemia in dairy cows based on an automated behavior activity monitor data. P. H. E. Trindade^{*1}, S. Borchardt², T. A. Burnett³, C. Gondro¹, and A. M. L. Madureira¹, ¹Michigan State University, East Lansing, Michigan, ²Free University Berlin, Berlin, Germany, ³University of Guelph, Ridgetown, ON, Canada.

The periparturient period is a physiological challenge with a high risk of subclinical hyperketonemia (SCK). We aimed to develop a machine learning algorithm to predict SCK in transition dairy cows using an automated activity monitor (AAM). Behavioral data (active, inactive, eating, rumination) were recorded minutely for 5,090 Holstein cows using an

AAM (Smarttag Neck) for 35 d precalving. Cows were monitored for SCK at 8 DIM using a ketone meter. Cows with BHB ≥ 1.2 mmol/L were diagnosed with SCK and treated with 250 mL of propylene glycol for 5 d. Health disorders, including retained placenta, metritis, milk fever, mastitis, or displaced abomasum within 30 DIM, were recorded. Cows were classified into 2 groups: healthy (HLT, $n = 3,471$) with no SCK or other health issues and cows with SCK with no other health problems within 30 DIM ($n = 1,619$). A random forest algorithm was developed using AAM behaviors as feature variables, with health status (HLT vs. SCK) as the target variable. Individual models were trained and tested for each time point (35, 28, 21, 14, 7, and 0 d before calving). To train the algorithm, 70% of the cows were randomly selected, while the remaining were used to evaluate predictive performance. The algorithm consisted of 1,001 trees with 2 randomly selected variables per tree and employed 5-fold cross-validation with 5 repetitions. Feature importance was calculated to assess each variable's contribution to the prediction. Predictive performance was evaluated based on accuracy, specificity, and sensitivity. The algorithm predicting HLT vs. SCK achieved high accuracy (91.43%–93.15%) and sensitivity (96.67%–97.79%) across all time points. Specificity ranged from 69.29% to 76.19%. Rumination, eating, and inactivity were the most important predictors, particularly at 14 d precalving, when algorithm performance was highest (93.15% accuracy, 76.19% specificity). Behavioral monitoring during the transition period could help predict SCK, supporting timely decisions to improve cow health, welfare, and farm efficiency.

Key Words: subclinical hyperketonemia, random forest, machine learning

2612 Evaluation of milk yield and liver functionality index of early-lactation Holstein cows diagnosed with concurrent hyperketonemia and hypoglycemia. G. F. M. Leão^{*}, M. Bugoni, L. S. Nogueira, N. P. G. Viana, and R. Almeida, Universidade Federal do Paraná, Curitiba, PR, Brazil.

The objective of this study was to characterize liver functionality index (LFI) of early-lactation dairy cows diagnosed with hyperketonemia (HK), hypoglycemia (HG), or concurrent HK and HG in 3 different Holstein herds. Data were collected between October 2023 and June 2024 from 3 farms located in Paraná State, Southern Brazil. Glucose and BHB concentrations were measured in whole blood using a handheld device (FreeStyle Optium Neo, Abbott Laboratories), from cows between 3 and 9 d postpartum. In total, 399 cows were categorized into 4 groups: NORM: BHB < 1.2 mmol/L and glucose > 2.2 mmol/L; $n = 273$), HK only (BHB ≥ 1.2 mmol/L; $n = 17$), HG only (glucose ≤ 2.2 mmol/L; $n = 92$), and concurrent HK and HG (HKHG; $n = 17$). Milk yield was daily recorded until 100 DIM. Serum samples were collected on days +3 and +28 related to calving, to determine metabolites albumin, cholesterol, and total bilirubin, 3 plasma biomarkers routinely synthesized by the liver, required to LFI calculation. Statistical analysis for those parameters was carried out in SAS using the MIXED procedure, with metabolic status (NORM, HK, HG, HKHG) category, parity, and herd as fixed effects. Average ($\pm$ SEM) for milk yield up to 100 DIM was higher ($P < 0.01$) for HG cows (50.87 ± 0.17) than the remaining categories (HK = 49.87 ± 0.29 ; HKHG = 49.73 ± 0.28 ; NORM = 49.39 ± 0.12). Moreover, LFI was lower ($P = 0.02$) for HK animals (-1.60 ± 0.72), intermediate for NORM (-0.48 ± 0.42), and higher for HKHG and HG cows (0.23 ± 0.76 and 0.60 ± 0.49 , respectively). In conclusion, postpartum HG Holstein cows showed a favorable combination of higher milk yield and adequate liver health.

Key Words: beta-hydroxybutyrate, ketosis, transition period

2613 Assessment of a rapid immune status test device following systemic or intrauterine LPS challenges in early-postpartum cows. T. C. Bruinje¹, C. Roeschmann², O. Willoughby², L. Campora², and S. G. Umaña Sedó³, ¹Dairy and Food Science, South Dakota State University, Brookings, SD, ²Population Medicine, University of Guelph, Guelph, ON, Canada, ³Large Animal Clinical Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA.

The objective was to assess changes in type 1/type 2 immune status using a rapid blood test device following intravenous (IV) or intrauterine (IU) lipopolysaccharide (LPS) challenges at 5 or 40 DIM. Healthy cows at 5 DIM were randomly assigned to receive IV-LPS (0.0625 µg/kg BW; n = 8), IU-LPS (100 µg of LPS in 20 mL saline; n = 8), or IU saline as control (CON; n = 7). At 40 DIM, different cows were randomized to IV-LPS (0.1 µg/kg BW; n = 6) or CON (n = 8), as those randomized for IU-LPS for a different study were not used here. Cows were fasted for 12 h after challenge. Serum samples obtained immediately before and at 12, 24, and 48 h after challenge were analyzed using a rapid handheld immune test device (D2Dx, CT-100, Nano Discovery Inc.). It estimates the type 1/type 2 immunity ratio based on a gold nanoparticle reaction with IgG, IgM, and complement proteins. Higher test scores (i.e., absorbance change) represent greater polarization of type 2 in relation to type 1 immunity. Data were analyzed separately for 5 and 40 DIM in linear regression models including the fixed effects of treatment, time, and their interaction, with parity and baseline score as covariates, and cow as the repeated measures subject. At 5 DIM, there was an interaction between treatment and time. The test score at 12 h was lower in IV-LPS (0.060 ± 0.006) than CON (0.081 ± 0.006; $P = 0.06$) or IU-LPS (0.089 ± 0.006; $P < 0.01$), but it was similar at 24 or 48 h. At 40 DIM, the test score was not affected by treatment, but it decreased from 12 (0.085 ± 0.005) to 48 h (0.069 ± 0.005; $P = 0.05$). The temporal changes in the test score regardless of treatment suggest that the experimental method (e.g., fasting) could have influenced the test. The IV-LPS treatment caused changes in clinical signs, rectal temperature, and serum acute phase proteins indicative of systemic inflammation (reported in a separate study). Here, this model resulted in detectable changes in the D2Dx test scores, indicating a greater type 1-biased immunity, 12 h after IV-LPS challenge at 5 DIM. However, that did not occur at 40 DIM. Further investigation is needed to validate the use of this device in health monitoring of postpartum dairy cows.

Key Words: immune function, inflammation, transition health

2614 Performance of a machine learning model for real-time prediction of health status of lactating cows. S. Agüero*, M. M. Perez, O. D. Reyes, O. Messi, S. H. An, and J. O. Giordano, Cornell University, Ithaca, New York.

The objective of this study was to evaluate the performance of an XGBoost model trained with behavioral, physiological, performance, environmental, and cow feature data for classifying cow health status (HS). The model predicted HS based on physical activity, resting, reticulo-rumen temperature, rumination and eating behavior, milk yield, conductivity and components, temperature and humidity index, parity, calving features, and stocking density. Daily at 5 a.m., the model generated a HS prediction [0 = no health disorder (HD); 1 = health disorder]. At 7 a.m., technicians blind to the prediction conducted clinical exams on cows from 3 to 11 DIM to classify cows (n = 625) as affected (HD = 1) or not (HD = 0) by metritis, mastitis, ketosis, indigestion, displaced abomasum, and pneumonia. Using each day a cow presented clinical signs of HD as a positive case (i.e., HD = 1), metrics of performance (%; 95% CI) were: sensitivity (Se) = 57 [52, 62], specificity = 81 [80, 82]; positive predictive value (PPV) = 20 [18, 22], negative predictive

Table 1 (Abstr. 2614). Sensitivity and PPV for an XGBoost algorithm trained to predict cow health status using fixed intervals before and after clinical diagnosis as positive cases

Day relative to CD	Se (%)	95% CI	PPV (%)	95% CI
-5 to 0	58	49, 67	21	16, 25
-3 to 0	55	46, 64	19	15, 24
-5 to 1	69	61, 78	24	20, 29
-5 to 3	81	73, 88	28	23, 33
-5 to 5	86	80, 92	30	25, 34
-3 to 1	67	58, 75	23	18, 27
-3 to 3	78	70, 86	27	22, 31
-3 to 5	83	76, 90	28	24, 33
0 to 3	75	68, 83	24	20, 29
0 to 5	81	73, 88	26	21, 31
-1 to 0	54	44, 63	18	14, 22
0 to 1	63	54, 72	20	16, 25
-1 to 1	66	57, 75	21	17, 26

value = 96 [95, 96], accuracy = 79 [78, 80], balanced accuracy = 69 [66, 72], F-1 Score = 29 [26, 32]. Sensitivity was also evaluated using fixed time intervals around clinical diagnosis of disease as a positive case (Table 1). Our findings suggest that the ability of an XGBoost algorithm trained on diverse sensor and nonsensor data to identify cows with HD was moderate when only days when cows presented clinical signs of disease were considered a positive case. Sensitivity and PPV can be improved substantially when all days within fixed intervals before and after clinical diagnosis are used as positive cases.

Key Words: machine learning, real-time, health

2615 Milk β-hydroxybutyrate, subclinical ketosis, and its association with fertility and milk constituents in Chilean grazing dairies. P. Melendez*, V. Pattarajinda, and O. Nekouei, Jockey Club College of Veterinary Medicine & Life Sciences, City University of Hong Kong, Hong Kong, SAR.

The objective of this study was to evaluate the association between β-hydroxybutyrate (BHB) concentrations in milk and other milk metabolites during the first 30 d in milk, as well as the relationship between BHB in milk and conception rate at first service (CRFS) in Holstein cattle under grazing management in southern Chile. A large dataset from 527 dairies, including 229,989 lactations between 2019 and 2023, was used. Dairies were of different sizes (50–2,000 cows) and used various reproductive management methods such as synchronization protocols, heat detection, and a combination of both. Milk measurements were made through a cooperative DHIA (Cooprinsem). The variables evaluated were milk production (kg), fat %, protein %, milk urea nitrogen (MUN; mg/dL), somatic cell counts (SCC; ×1,000/mL), and BHB (mmol/L) on the first test day (T1; 21 ± 10 d in milk). Milk BHB was converted to blood BHB using the equation: blood BHB = 0.49 + 5.55 × milk BHB. The CRFS for the entire population was 55.9%. Milk BHB in pregnant and open cows was 0.043 ± 0.05 mmol/L (blood BHB = 0.728 ± 0.76) and 0.045 ± 0.05 (blood BHB = 0.740 ± 0.76), respectively ($P < 0.001$). Using the cut-off of 1.2 mmol/L of BHB in blood (milk BHB = 0.128 mmol/L), the prevalence of subclinical ketosis (SCK) during the first 30 DIM was 5.56%. Using this information, milk production, solids, MUN, SCC at T1, and CRFS were compared between SCK and non-SCK cows. Milk yield, solids, MUN, and log SCC were analyzed by GLM ANOVA correcting by farm and year. The CRFS was compared by logistic regression also correcting by farm and year. Cows with SCK

Table 1 (Abstr. 2615).

Subclinical ketosis	SCK, %	Milk ¹ , kg	Fat, %	Protein, %	MUN, mg/dL	SCC, ×1,000/mL	CRFS, %
No (n = 232,920)	—	26.2 ^a (9.09)	4.00 ^a (1.12)	3.55 ^a (0.61)	12.9 (5.7)	310.6 ^a (969)	56.1 ^a
Yes (n = 12,794)	5.56	25.0 ^b (8.76)	4.68 ^b (1.28)	3.47 ^b (0.50)	12.7 (5.6)	621.8 ^b (1,628)	53.5 ^b

^{a,b}*P* < 0.001¹The values in parentheses are SD.

had lower CRFS than non-SCK cows. Cows without SCK were 1.11 (95% CI: 1.07–1.15) times more likely to conceive at first service than cows with SCK. On average, cows without SCK had higher milk yield and protein% but lower fat% and SCC than cows with SCK at T1. These results suggest that the milk BHB at T1 can be used to predict fertility and lactational performance of Holstein cattle handled under grazing. Results of the experiment are shown in Table 1.

Key Words: BHB, ketosis, grazing

2616 Milk β-hydroxybutyrate, subclinical ketosis, and its association with fertility and milk constituents in confinement Chilean dairies. P. Melendez*, V. Pattarajinda, and O. Nekouei, *Jockey Club College of Veterinary Medicine & Life Sciences, City University of Hong Kong, Hong Kong, SAR.*

The objective of this study was to evaluate the association between β-hydroxybutyrate (BHB) concentrations in milk and other milk metabolites during the first 30 d in milk, as well as the relationship between BHB in milk and conception rate at first service (CRFS) in Holstein cattle under confinement in central Chile. A large data set was analyzed from 26 dairies, including 18,016 lactations, between 2019 and 2023. Dairies were of different sizes (200–2,350 cows), and all used synchronization of ovulation-timed AI protocols. Milk measurements were made through a cooperative DHIA (Cooprinsem). The variables evaluated were milk production (kg), fat %, protein %, milk urea nitrogen (MUN; mg/dL), somatic cell counts (SCC; ×1,000/mL), and BHB (mmol/L) on the first test day (T1; 19 ± 8 d in milk). Milk BHB was equivalent to blood BHB using the equation: blood BHB = 0.49 + 5.55 × milk BHB. The CRFS for the entire population was 45.6%. Milk BHB in pregnant and open cows was 0.052 ± 0.05 mmol/L (blood BHB = 0.783 ± 0.76) and 0.056 ± 0.05 (blood BHB = 0.80 ± 0.76), respectively (*P* < 0.01). Using the cut-off of 1.2 mmol/L of BHB in blood (milk BHB = 0.128 mmol/L), the prevalence of subclinical ketosis (SCK) during the first 30 DIM was 8.38%. Milk production, solids, MUN, SCC at T1, and CRFS were compared between cows with and without SCK. Milk yield, solids, MUN, and log SCC were analyzed by GLM ANOVA correcting by farm and year. The CRFS was compared by logistic regression, also correcting by farm and year. Cows without SCK were 1.14 (95% CI: 1.02–1.26) times more likely to conceive at first service than cows with SCK. On average, cows without SCK had higher milk yield, protein %, and MUN, but lower fat % and SCC than cows with SCK. It is concluded that cows with SCK during the first 30 DIM had lower CRFS, produced less milk

at T1, and had poorer udder health than normal cows without SCK in confinement Holstein dairies managed under Chilean conditions. Results of the experiment are shown in Table 1.

Key Words: BHB, ketosis, confinement

2617 Associations between prepartum urine pH and periparturient blood calcium concentrations in multiparous Holstein cows. J. P. Goff*, P. Melendez², T. R. Overton³, J. K. Drackley⁴, S. J. LeBlanc⁵, R. Couto Serrenho⁵, and J. E. P. Santos⁶, ¹*Iowa State University, Ames, IA*, ²*City University of Hong Kong, Hong Kong, China*, ³*Cornell University, Ithaca, NY*, ⁴*University of Illinois, Urbana, IL*, ⁵*University of Guelph, Guelph, ON, Canada*, ⁶*University of Florida, Gainesville, FL.*

Metabolic alkalosis, induced by excessive prepartum diet cations, impairs calcium homeostasis in periparturient cows. Adding anions to the diet acidifies the cow's blood, improving calcium homeostasis. Urine pH reflects blood pH and is easier to measure and is used to assess diet DCAD sufficiency. The degree to which urine should be acidified is debated. Individual prepartum urine pH and periparturient blood Ca concentrations determined in 660 multiparous cows from 9 studies were analyzed to determine the urine pH associated with the lowest and highest periparturient blood Ca concentrations. Means of the lowest blood Ca concentration (Ca nadir) observed in cows within 6 urine pH categories (≤5.75, 5.76 to 6.25, 6.26 to 6.75, 6.76 to 7.25, 7.26 to 7.75, and ≥7.76) were determined. The association between prepartum urine pH and Ca nadir was examined using mixed-effects models with the fixed effects of the linear covariates of urine pH, the quadratic covariates of urine pH, cow parity, and their interactions, and the random effect of experimental study. Data were analyzed with all 660 cows, and then stratified into cows entering their 2nd (n = 298), 3rd (n = 195), or 4th and greater parity (n = 167). Across all parity groups the lowest mean Ca nadir was observed when urine pH was >7.76. An increase in Ca nadir was seen across all 660 multiparous cows when urine pH was below 7.26. Further reductions in urine pH did not increase Ca nadir significantly, especially for cows in their 2nd or 3rd parity. Among >4th parity cows, urine pH above 7.25 or below 5.75 were associated with significantly lower Ca nadir than when urine pH was between 6.26 and 6.75. The incidence of subclinical hypocalcemia (SCH), defined as a blood Ca concentration below 2.00 mM, was also greatest in cows with urine pH above 7.75. Parity had a larger effect on Ca nadir than did urine pH. The largest increase in Ca nadir is made by reducing the degree of

Table 1 (Abstr. 2616).

Subclinical ketosis	SCK, %	Milk ¹ , kg	Fat, %	Protein, %	MUN, mg/dL	SCC, ×1,000/mL	SCC, LS ²	CRFS, %
No (n = 16,506)	—	37.5 ^a (11.9)	4.05 ^a (1.25)	3.49 ^a (0.74)	13.2 ^a (4.8)	298 ^a (871)	4.6 ^a (6.1)	46.1 ^a
Yes (n = 1,510)	8.38	36.8 ^b (11.9)	4.60 ^b (1.41)	3.36 ^b (0.44)	12.8 ^b (4.6)	540 ^b (1,394)	5.4 ^b (6.8)	42.9 ^b

^{a,b}*P* < 0.01.¹The values in parentheses are SD.²LS = linear score.

alkalinity of the urine to levels below 7.75 for all but the oldest cows. For 4th and greater parity cows urine pH below 7.25 was associated with improved Ca nadir.

Key Words: hypocalcemia, urine pH, DCAD

2618 Comparison of liver ultrasound measurement and blood metabolites in lactating Jersey cows with different abdominal fat and body condition scores. V. Pattarajinda, C. H. Lau, E. Done, J. Ip, and P. Melendez*, *Jockey Club College of Veterinary Medicine & Life Sciences, City University of Hong Kong, Hong Kong, SAR.*

This study aimed to estimate abdominal fat by ultrasound and compared liver traits, and blood metabolites between cows with high and low abdominal fat and between cows with high and low BCS. This study was conducted at the City University of Hong Kong dairy. Twenty lactating healthy jersey cows were randomly selected and a transcutaneous ultrasonography on 6 anatomical external body areas was carried out. A blood sample was obtained from the tail blood plexus. In addition, BCS (scale of 1 to 5 and 1/4 unit increments) was conducted by the same evaluator. Ultrasound measurements were used to estimate the amount of total abdominal fat (TAF), omental (OF), retroperitoneal (RF), and mesenteric fat (MF). Cows were classified as high TAF ($n = 10$; 25.9 kg fat, 113 DIM, 3.375 median BCS) and low TAF ($n = 10$; 14.7 kg fat, 149 DIM, 3.125 median BCS). An evaluation of liver traits by ultrasound was also conducted. Depth of liver (DL), depth of portal vein (DPV), portal vein area, portal vein perimeter, portal vein diameter (PVD), depth of perivisceral adipose tissue (DPAT), and thickness of perivisceral adipose tissue (TPAT) were assessed. As a second criterion cows were also classified as high (median 3.5) and low BCS (median 2.75) and compared for liver traits and blood metabolites. Both outcomes were analyzed by GLM ANOVA, correcting for DIM. Within the abdominal fat criterion, cows with low MF tended to have a larger portal vein area than cows with high MF (5.43 vs. 4.57 cm², respectively; $P = 0.06$). Cows with high TAF and OM, had higher TPAT than cows with low TAF and OM ($P \leq 0.01$). Blood metabolites were not different between cows with high and low TAF. Within the BCS criterion, the group with low BCS had a higher portal vein area than the high BCS group (5.37 vs. 4.44 cm², respectively; $P = 0.05$). Also, cows with low BCS had higher Ca concentrations (2.59 vs 2.42 mmol/L, SD = 0.17, respectively; $P \leq 0.05$) and higher urea concentrations (8.1 vs 6.5 mmol/L, SD = 1.76, respectively; $P \leq 0.05$) than cows with high BCS. In conclusion, cows with more abdominal adiposity and higher BCS had a lower portal vein area and a higher amount of perivisceral adipose tissue.

Key Words: abdominal fat, liver traits, BCS

2619 Infrared thermography and ultrasonography for the prediction of abdominal adiposity in lactating Jersey dairy cows. C. H. Lau, V. Pattarajinda, E. Done, J. Ip, and P. Melendez*, *Jockey Club College of Veterinary Medicine & Life Sciences, City University of Hong Kong, Hong Kong, SAR.*

Excessive abdominal fat in dairy cows increases the risk of metabolic diseases during the transition period. Body condition score is only moderately related to abdominal fat, limiting its efficacy as an early disease detection method. This study aimed to compare the infrared thermography (IRT) and blood metabolites between animals with high and low abdominal fat in Jersey cows. The study was conducted at the dairy farm of City University of Hong Kong. Twenty lactating healthy Jersey cows were randomly selected and a transcutaneous ultrasonography on 6 anatomical external body areas was carried out. The BCS

(scale of 1 to 5 and 1/4 unit increments) was conducted by the same evaluator. Ultrasound measurements were used to estimate the amount of total abdominal fat (TAF), omental, retroperitoneal, and mesenteric fat (MF). Cows were classified as high TAF ($n = 10$; 25.9 kg fat, 113 DIM, 3.375 median BCS) and low TAF ($n = 10$; 14.7 kg fat, 149 DIM, 3.125 median BCS). Abdominal skin temperature at 5 points on the right flank was measured by IRT. A blood metabolic profile was performed at the veterinary diagnostic laboratory. Body condition score was compared by the Kruskal–Wallis test, and blood metabolite, abdominal fat, and IRT were compared by GLM ANOVA correcting by BCS and DIM. Body condition score was not different between groups ($P = 0.38$), but TAF was ($P \leq 0.001$). Cows with higher MF had a trend ($P = 0.14$) for lower mean temperature ($29.52^{\circ}\text{C} \pm 0.94^{\circ}\text{C}$) at the right ventral flank than cows with lower MF ($30.08^{\circ}\text{C} \pm 0.67^{\circ}\text{C}$). Cows with higher MF showed lower calcium ($P = 0.006$), bicarbonate ($P = 0.05$), and urea ($P = 0.013$) than cows with low MF, but the values were within the normal ranges. Of the 6 anatomical points evaluated by ultrasound, measurement of the central area of the right paralumbar fossa (PF) was most associated with differences in IRT. Cows with a higher fat on PF had a lower temperature on the ventral flank (29.42°C vs 30.11°C ; $P = 0.06$) and lower urea ($P = 0.011$) than cows with lower fat. It is concluded that cows with greater abdominal fat tended to have a lower IRT in the right ventral flank than cows with less abdominal fat, suggesting that the fat may act as an insulation tissue.

Key Words: abdominal fat, infrared thermography, ultrasound

2620 Temporal pattern of performance, metabolism, and inflammation relative to disease diagnosis in early-lactation Holstein cows. G. J. Combs*, B. M. Goetz, A. Nayeri, N. C. Upah, T. A. Flemming, E. J. Mayorga, B. M. Buol, L. C. Bombeck, and L. H. Baumgard, *Department of Animal Science, Iowa State University, Ames, IA.*

Objectives were to elucidate temporal patterns of key performance indicators surrounding disease onset during lactation. Primi- and multiparous Holstein cows ($n = 294$) from 4 transition cow trials were retrospectively classified as healthy (HEL; no diagnosis; $n = 168$) or sick (SK; ≥ 1 diagnosis and treated per veterinarian recommendations; $n = 126$). Diagnoses were classified by symptoms and SK values were analyzed as a percentage of HEL means at equivalent DIM within study. Metabolic ($n = 158$ cows), and inflammatory data ($n = 96$ cows) were grouped by days from diagnosis (DFD); all data were analyzed using repeated measures of DFD using PROC MIXED with fixed effects of study and diagnosis. Sick cows had a 22% reduction in DMI from -4 to -1 DFD ($P < 0.01$). Milk yield (MY) decreased 12% in SK at -1 DFD and reached a nadir at 1 DFD ($P < 0.01$). Rumination time (RT; $n = 63$ cows) was less in SK from -14 DFD (15%; $P < 0.01$) and decreased further from -2 to 1 DFD (38% reduction at nadir). Milk yield remained decreased through 7 DFD ($P \leq 0.02$), while RT and DMI remained depressed until 12 DFD ($P < 0.01$). A diagnosis $\times$ DFD interaction ($P < 0.01$) occurred where diagnoses with ill-defined etiology (i.e., nonspecific sickness behavior) or lameness did not reduce MY or DMI around diagnosis, and clinical hyperketonemia mildly reduced DMI before diagnosis with no change in MY. In contrast, infection (e.g., mastitis, metritis), displaced abomasum, and clinical milk fever markedly decreased DMI, MY, and RT pre- and postdiagnosis and were the subject of metabolic inquiry. Nonesterified fatty acids and BHB were greater in SK at -2 ± 2 and 2 ± 2 DFD, respectively (25% and 88%; $P \leq 0.02$). Haptoglobin (Hp) was greater at -5 ± 2 DFD in SK (5.2-fold) and LPS-binding protein (LBP) tended ($P < 0.07$) to increase at -2 ± 2 DFD (20%). The LBP remained elevated through 2 ± 2 DFD, while Hp was not different at -2 ± 2 DFD

Table 1 (Abstr. 2621).

Response	Treatment				SEM	P-value				
	CHOL1	CHOL3	CAL1	CAL3		Source (S)	Amount (A)	Metritis (M)	S × A	S × A × M*
Prepartum DMI, kg/d					0.6	0.78	0.03	0.75	0.02	0.05
Non-MET	11.7	11.2	11.3	11.2						
MET	12.9 ^a	9.5 ^b	11.0 ^{ab}	11.3 ^{ab}						
Milk yield, kg/d					2.5	0.20	0.72	0.01	<.0001	0.05
Non-Met	44.1	43.6	41.7	43.9						
Met	34.2 ^{ab}	26.2 ^b	31.4 ^b	40.3 ^a						
Calcidiol, ¹ ng/mL					6.3	<.0001	<.0001	0.21	<.0001	0.15
Non-Met	58.5	59.9	105	206						
Met	62.9	64.3	100	227						
Ca, ² mM					0.04	0.36	0.94	<0.001	0.29	0.29
Non-Met	2.18	2.16	2.20	2.18						
Met	2.08	2.05	2.05	2.13						
Hp, ³ µg/mL					132	0.09	0.54	<0.0001	0.47	0.03
Non-Met	299	278	209	364						
Met	1,188 ^a	1,143 ^a	1,265 ^a	695 ^b						

¹Measured in serum at d 0 postpartum.

²Measured in serum at d 2, 3, 4, 5, 8 and 11 postpartum.

³Measured in serum at d 0, 3, 8 and 15 postpartum.

*If the interaction between S × A × M was significant, the PROC-ILM statement in SAS was used to slice metritis for assigning superscript letters within non-MET and MET according to pairwise comparisons.

but elevated postdiagnosis (6.1-fold; $P \leq 0.03$). Serum amyloid A was not different over time ($P > 0.21$). In summary, disease onset markedly decreased DMI at least 4 d before diagnosis coinciding with marked increases in LBP and Hp, and this preceded declines in MY relative to healthy counterparts.

Key Words: infection

2621 Effect of source and amount of vitamin D supplementation on performance and inflammation of transition multiparous cows according to metritis. A. Fraz*, M. B. Poindexter, and C. D. Nelson, *University of Florida, Gainesville, FL.*

Post hoc analyses of a previous experiment were conducted to evaluate the effects of supplementing prepartum cholecalciferol (CHOL) compared with calcidiol (CAL) at 1 or 3 mg/day on performance and inflammation of transition cows in the absence or presence of metritis. Parous cows (n = 126) were blocked by previous lactation milk yield and assigned vitamin D treatments as CHOL at 1 mg/day (CHOL1) or 3 mg/day (CHOL3) and CAL at 1 mg/day (CAL1) or 3 mg/day (CAL3). Vitamin D treatments were fed for 24 d prepartum. Cows were evaluated for metritis on d 4, 7, and 12 postpartum by metricheck. Cows not diagnosed with metritis were classified as non-MET and those diagnosed with metritis were classified as MET. Data were analyzed for the effects of vitamin D treatments, metritis, and interactions among treatments and metritis. Daily prepartum dry matter intake and milk yield up to 42 DIM were recorded. Data analyses were performed using PROC MIXED in SAS by incorporating the fixed effects of source, amount, metritis and all possible 2-way interactions and 3-way interaction between source, amount and metritis. If 3-way interaction (source × amount × metritis) was $P < 0.05$, PROC-ILM statement in SAS was used to slice metritis for assigning superscript letters within non-MET and MET according to pairwise comparisons. Results are in Table 1. In cows diagnosed with metritis, CHOL3 supplementation led to negative effects on feed intake

and milk yield, while CAL3 supplementation enhanced milk yield and lowered haptoglobin concentrations.

Key Words: calcidiol, metritis, inflammation

2622 Effect of length of stay in postpartum pen and genomic net merit on BCS change, health, and culling of Holstein dairy cows. L. Prada Gutierrez*, G. Schuenemann¹, S. M. Bellinzoni¹, J. Lakritz², A. Garcia Guerra³, and G. M. Schuenemann¹, *¹Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, ²Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH, ³Department of Animal Sciences, The Ohio State University, Columbus, OH.*

The objective was to assess the effect of length of stay in postpartum pen (LSPP) and genomic lifetime net merit (NM\$) on BCS change, health, and culling of Holstein dairy cows. A total of 817 multiparous Holstein cows from 2 Ohio dairy herds were enrolled in monthly cohorts of 20 to 36 cows at 21 ± 3 d before expected calving date. At calving, cows were randomly assigned into 1 of 3 LSPP groups: 7 (n = 260), 14 (n = 283) or 21 d in milk (DIM; n = 274). Once each LSPP group was completed, lactating cows were moved into the high lactation group. Cows were fed a total mixed ration formulated to meet nutritional requirements for prepartum, postpartum, and high lactation diets. Health events within 30 DIM were recorded and cows were categorized into 1 of 4 groups: (1) nondiseased (ND); (2) cows with only ketosis (KET; BHB ≥ 1.2 mmol/L); (3) sick cows experiencing ≥ 1 health conditions, but without ketosis (SICK); or (4) cows with ketosis plus at least one other health condition (KET+). Lifetime NM\$ was obtained for a subset of cows (n = 406). Calcium (Ca) was assessed within 48 h after calving and BCS change (BCSΔ; 5-point scale) was assessed between calving and 28 ± 3 DIM. Culling within 60 DIM (CULL60) was recorded. Data were analyzed using MIXED or GLIMMIX procedures of SAS. Parity was included as a fixed effect and NM\$ was included as a covariate. Farm was

Table 1 (Abstr. 2622).

Item	Length of stay in postpartum pen relative to calving, d			
	7	14	21	P-value
Lactation, n	2.76 ± 0.05	2.84 ± 0.04	2.84 ± 0.4	0.49
BCSA	0.31 ± 0.03	0.36 ± 0.03	0.34 ± 0.03	0.32
Ca <8.0 mg/dL, %	43.03 ± 3.35	36.70 ± 3.20	37.44 ± 3.22	0.33
CULL60, %	5.65 ± 1.32	6.06 ± 1.25	3.67 ± 1.27	0.36
ND, %	53.91 ± 3.08	48.78 ± 2.93	54.50 ± 2.98	0.31
KET, %	5.38 ± 1.47	6.36 ± 1.41	6.20 ± 1.43	0.87
KET+, %	8.90 ± 1.78	10.34 ± 1.69	10.26 ± 1.72	0.80
SICK, %	31.14 ± 2.90	34.08 ± 2.76	28.61 ± 2.80	0.37

included as a random effect. Least squares means ± SEM were reported. Parity, BCSA, Ca, ND, KET, KET+, SICK, and CULL60 did not differ among LSPP groups (Table 1). However, cows with NM\$ > 450 had increased ND by 17.57% and reduced KET, KET+, and CULL60 by 68.3%, 62.6%, and 74%, respectively, compared with cows with NM\$ ≤ 450 ($P < 0.05$). Although BCSA, health, and CULL60 did not differ by LSPP groups, these findings show that cows with NM\$ > 450 have improved overall health and survival.

Key Words: transition dairy cows, lifetime net merit, health

2623 Transition cows and cows with gastrointestinal or metabolic disease have increased loads of multiple enteric pathogens.

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Higher pathogen loads and coinfection in dairy cows impact health and decrease productivity. The objective of this study was to determine how quantities of common gastrointestinal (GI) pathogens differed by health status or phase of lactation (LACT) in dairy cows across the United States. Rectal swab samples (Copan FecalSwab) were collected from cows and heifers (n = 2,026) from 88 commercial dairy farms across 19 states between 2018 and 2024. Genomic DNA was extracted from rectal swab tips (Qiagen PowerFecal Pro kit) and a qPCR panel was used to quantify common GI microbial virulence and marker genes via absolute quantification with standard curves. Cows with GI or metabolic disease (diarrhea, ketosis, displaced abomasum, and off-feed events) were categorized as sick (n = 636 vs. healthy n = 1,390). Average gene quantities were evaluated using Kruskal–Wallis test, followed by Dunn's test with Holm correction for pairwise comparisons by stage of LACT (fresh = 0–21 DIM, early-LACT = 22–100 DIM, mid-LACT = 101–200 DIM, late-LACT = 201–305 DIM, and dry). Sick cows had increased ($P < 0.001$) total *E. coli* ZnT and O157 marker, as well as the *E. coli* virulence genes *eaeA* ($P = 0.005$) and enteroaggregative stable toxin *EAST1* ($P < 0.001$) compared with healthy cows. *Clostridium perfringens* α toxin gene *cpa* ($P < 0.001$) and *Salmonella enterica* *invA* gene ($P < 0.001$) were also higher in sick compared with healthy cows. Independently of health status, late-LACT, dry, and fresh cows had higher ($P < 0.002$) total *E. coli* ZnT than early- and mid-LACT. The *E. coli* marker O157 was also increased ($P = 0.002$) in dry and fresh, while dry cows had the lowest ($P = 0.004$) quantities of stable toxin gene *STa*. The *E. coli* virulence gene *eaeA* was higher ($P < 0.001$) in fresh and early-LACT, and Shiga toxins 1 ($P = 0.017$) and 2, *EAST1*, and *C. perfringens* *cpa* were increased ($P < 0.001$) only in fresh cows. Fresh and dry cows also had higher quantities of the bovine coccidia *Eimeria bovis* ($P < 0.001$). In conclusion, loads of multiple pathogens

were increased in sick cows, and cows during the transition period had the highest quantities of pathogenic *E. coli*, *C. perfringens*, and *E. bovis*.

Key Words: coinfection, transition period, gut pathogens

2624 Anti-inflammatory treatment at dry-off: Effects on metabolic status in high-priority cow groups—Part I. R. Sorto^{*1}, R. Landrum¹, M. Molins², L. Periconi¹, M. Marinelli¹, K. J. Harvatine², T. Ott², E. Hovingh¹, and A. A. Barragan¹, ¹Department of Veterinary and Biomedical Sciences, Penn State University, State College, PA, ²Department of Animal Sciences, Penn State University, State College, PA.

Drying cows off is a vital practice in modern dairy farming. However, this practice may be associated with stress and inflammation in some groups of cows, predisposing them to diseases and poor performance. The objective of this study was to assess the effects of acetylsalicylic acid administration at dry-off on the metabolic status (i.e., nonesterified fatty acid concentration [NEFA], and β -hydroxybutyrate concentration [BHB]) during the first 5 d post-dry-off. On the day of dry-off cows were blocked by parity (primiparous [PRIM]; multiparous [MULT]) and randomly allocated to one of 2 treatments: aspirin (ASA; n = 77): receive an oral treatment with 4 acetylsalicylic acid boluses (125 g/cow); or placebo (PLC; n = 80): receive an oral treatment with 4 gelatin capsules filled with water. The BCS was measured at dry-off to group cows in BCS categories (BCSDRYCAT; optimal [OPT] = 2.75–3.5 pts.; over-conditioned [OVERC] = 3.75–5 pts.). Milk samples were collected at dry-off in a randomly selected subgroup of cows (ASA n = 28, PLC n = 23) to measure SCC, and group cows into SCC categories (SCCDRYCAT; LOWSCC <200,000 cells/mL; [n = 31], HIGHSCC ≥200,000 cells/mL; [n = 20]). Blood samples were collected daily for the first 5 d after dry-off for assessment of NEFA and BHB. The data were analyzed using the UNIVARIATE and MIXED procedures of SAS as a randomized complete block design. A treatment-by-parity interaction was observed, where MULT cows treated with ASA tended to have lower NEFA concentrations in the first 5 d post dry-off (ASA = 180.3 mEq/L, 95% CI: 166.6–195.1; PLC = 196.78 mEq/L, 95% CI: 182.8–211.8; $P = 0.10$). Within the SCC cohort, there was interaction between treatment and SCCDRYCAT ($P = 0.004$). The HIGHSCC cows treated with ASA had lower NEFA concentrations during the first week post dry-off. Additionally, a 3-way interaction (treatment × parity × day) was observed for BHB concentrations, where primiparous cows treated with ASA had higher BHB concentrations on d 2, 3, and 4 compared with PLC PRIM cows ($P = 0.003$). On the other hand, HIGHSCC cows treated with ASA had lower BHB concentrations, whereas PLC LOWSCC cows had higher BHB concentrations, compared with PLC HIGHSCC and ASA LOWSCC, respectively ($P = 0.01$). These findings suggest that ASA administration at dry-off may benefit the metabolic status in the first week after dry-off, particularly in MULT and HIGHSCC cows.

Key Words: dry-off, anti-inflammatory treatment, metabolic status

2625 Anti-inflammatory treatment at dry-off: Effects on milk yield, health, and reproductive performance in Holstein dairy cows. R. Landrum^{*1}, R. Sorto¹, N. Campos¹, L. Periconi¹, M. Molins², M. Marinelli¹, K. J. Harvatine², T. Ott², E. Hovingh¹, and A. A. Barragan¹, ¹Department of Veterinary and Biomedical Sciences, Penn State University, State College, PA, ²Department of Animal Sciences, Penn State University, State College, PA.

The objective of this study was to assess the effects of acetylsalicylic acid administration at dry-off on milk yield, clinical health events, and

reproductive performance in the subsequent lactation in Holstein dairy cows. On the day of dry-off, cows were blocked by parity (primiparous [PRIM]; multiparous [MULT]) and randomly allocated to one of 2 treatments: ASA (n = 168): receive an oral treatment with 4 acetylsalicylic acid boluses (125 g/cow); or PLC (n = 168): receive an oral treatment with 4 gelatin capsules filled with water. The BCS was measured at dry-off to group cows in BCS categories (BCSDRYCAT; optimal [OPT] = 2.75–3.5 pts.; over-conditioned [OVERC] = 3.75–5 pts.). Health events (≤ 60 DIM), daily milk yield (≤ 112 DIM), and reproductive performance (≤ 120 DIM) data were collected from on-farm computer records. The data were analyzed using the UNIVARIATE, MIXED, GLIMMIX, and LIFETEST SAS procedures as a randomized complete block design. Although cows treated with ASA produced less milk than those in the PLC group (ASA = 47.45 ± 0.47 kg/day; PLC = 48.87 ± 0.47 kg/day; $P = 0.02$), an interaction between treatment and BCSDRYCAT was observed, where PLC-OPT cows produced more milk than ASA OPT cows (ASA = 46.88 ± 0.77 kg/day; PLC = 49.44 ± 0.73 kg/day; $P = 0.01$). Regarding health, ASA cows had a lower incidence of clinical metritis than PLC cows (ASA = $1.88\% \pm 1.03\%$; PLC = $6.61\% \pm 2.12\%$; $P = 0.03$). Similarly, there was a tendency for treatment by parity interaction, where ASA MULT cows had a lower incidence of calving disorders compared with PLC MULT cows (ASA MULT = $6.24\% \pm 2.54\%$; PLC MULT = $13.59\% \pm 3.83\%$; $P = 0.08$). In terms of reproductive performance, ASA PRIM cows tended ($P = 0.05$) to have a higher pregnancy rate at first service than those receiving the placebo (ASA PRIM = $54.58\% \pm 12.85\%$; PLC PRIM = $26.8\% \pm 11.1\%$). Furthermore, ASA PRIM cows required 12 d less to get pregnant compared with PLC PRIM group ($P = 0.03$). Although dry-off ASA treatment decreased milk yield in OPT cows, treatment may improve overall health and fertility in early lactation.

Key Words: dry-off, anti-inflammatory treatment, health and performance

2626 Characterization of physiological, behavioral, health, and performance parameters in dairy cows with automated health alerts and no evident clinical signs of disease in early lactation. C. Rial*, S. Tompkins, A. S. Jacome-Aucay, N. A. López-Núñez, and J. O. Giordano, *Department of Animal Science, Cornell University, Ithaca, NY*.

This observational study aimed to characterize physiological, behavioral, health, and performance parameters in cows with automated health alerts (HA) and no clinical health disorders (CHD) diagnosed from 2 to 14 d in milk (DIM). Lactating Holstein cows (n = 120) were fitted with a neck-attached sensor (SenseHub) that monitored rumination time (RT) and activity (ACT) and generated a health index score (HIS). AHA was defined as HIS < 86 arbitrary units, daily RT < 250 min, or both. Milk yield (MY) was recorded thrice daily using automated meters (Dematron 75). Cows underwent clinical examinations and were sampled every other day from 2 to 10 and at 14 DIM for somatic cell count (SCC), vaginal discharge score (VDS, 0–5), rectal temperature (ReT), lameness score (LS; 1–5), and blood β -hydroxybutyrate (BHB) concentration. At 15 DIM, cows were classified in groups HA-NCHD (n = 25, HA without CHD), HA-CHD (n = 16, HA with CHD), NoHA-NCHD (n = 68, no HA or CHD), and NoHA-CHD (n = 11, CHD without HA). Data were analyzed using logistic regression and ANOVA with or without repeated measures. From 2 to 21 DIM, HA-NCHD cows had less ($P < 0.05$) RT, ACT, and MY than NoHA-NCHD cows but greater RT, ACT, HIS, and MY than HA-CHD and NoHA-CHD cows. Total MY (kg) at 21 DIM was lower ($P < 0.01$) in HA-CHD cows (564 ± 46) than in HA-NCHD (837 ± 36), NoHA-CHD (786 ± 56), and NoHA-NCHD (890 ± 24) cows. Cows in HA-NCHD had lower ($P < 0.01$) ReT at 4 and 10

DIM than HA-CHD and NoHA-NCHD cows but did not differ from NoHA-NCHD cows. Concentrations of BHB were greater ($P < 0.01$) in HA-CHD cows than in NoHA-NCHD, HA-NCHD, and NoHA-CHD cows. Greater ($P < 0.01$) VDS was observed for HA-CHD (3.4 ± 0.1) and NoHA-CHD (3.4 ± 0.2) cows than for NoHA-NCHD (2.8 ± 0.1) and HA-NCHD (2.9 ± 0.1) cows. No differences were observed for SCC or LS. Cows with HA but no evident clinical signs of disease may represent a distinct subgroup with unique behavior and physiological features and performance levels. Automated behavior monitoring may help identify cows with no evident clinical signs but at risk of reduced performance.

Key Words: rumination, health monitoring, sensor

2627 Comparing immune cell populations between first-lactation dairy cows that did or did not receive immunotherapy at dry-off using flow cytometry. E. Leonard*, S. Wang², B. Crooker³, S. Dow⁴, and L. Caixeta¹, ¹Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, MN, ²Department of Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, MN, ³Department of Animal Science, University of Minnesota, Saint Paul, MN, ⁴Department of Clinical Sciences, Colorado State University, Fort Collins, CO.

Mastitis on dairy farms is commonly controlled using antibiotics, but concerns over antimicrobial stewardship are rising. Utilizing immunotherapeutic agents to upregulate the innate immune system in the mammary gland could serve as an alternative. A liposome-toll-like receptor (TLR) agonist (LTC) immune stimulant generates synergistic immune activation by combining TLR3 and TLR9 agonists with charged liposomes and a mucosal adhesive agent. Our objective was to determine whether LTC administration at dry-off alters immune cell populations in the mammary gland. Eight first-lactation Holstein cows were randomly assigned to receive a 10-mL intramammary infusion of either treatment (TRT; 0.6% LTC; n = 13 quarters) or control (CON; Tris-buffered solution with 10% sucrose, pH 7.2; n = 13 quarters). All quarters that were free of an intramammary infection (SCC < 200,000 cells/mL and culture-negative) were enrolled as experimental units in the study. Teat cistern cells were collected using a cytobrush (Medscand Cytobrush Plus) at 0 h (pretreatment) and 12 h post-treatment. Cells were stained with the conjugated antibodies CD14:SBV790 and CD11b:AF647 (Bio-Rad Laboratories, Inc.) and analyzed using flow cytometry (FACS Celesta). Expression of CD11b and CD14 were used to identify monocytes/macrophages (CD11b+CD14+) and neutrophils (CD11b+CD14-). A repeated measures ANOVA was conducted to evaluate the effects of LTC and time (0 h vs. 12 h) on the geometric mean fluorescence intensity of CD11b and CD14 expression in CD11b+CD14- and CD11b+CD14+ immune cell populations. A significant effect of time was observed for CD11b+CD14- cells ($P < 0.01$) and CD11b+CD14+ cells ($P < 0.01$), indicating an overall increase in marker expression at 12 h. However, no effects of treatment ($P > 0.33$) or treatment-by-time interactions ($P > 0.36$) were observed. These findings suggest that LTC did not significantly alter CD11b or CD14 expression over time. It is possible that the immune response to dry-off alone was sufficient to induce changes in marker expression, potentially masking any additional effect of LTC. Future research on alternative time points or functional assays may provide further insight into the therapeutic potential of LTC.

Key Words: innate immunity, mastitis, antibiotic alternative

2628 Early postpartum markers for purulent vaginal discharge and endometritis. C. F. Roeschmann*, E. Morrison¹, E. S. Ribeiro², R. Couto Serrenho¹, and S. J. LeBlanc¹, ¹Department of Population

This prospective study evaluated the association of blood biomarkers in the first 2 wk postpartum with purulent vaginal discharge (PVD) and endometritis near the end of the voluntary waiting period (VWP). Data included 551 cows from 5 herds in Ontario. Serum haptoglobin (Hp) and calcium (Ca) were measured at 3 and 6 (± 2) DIM, and blood β -hydroxybutyrate (BHB) at 3, 6, 10, and 14 DIM. Dystocia, twins, stillbirth, milk fever, and retained placenta were combined into a 2-level categorical variable, calving problems (CP). Assessments of PVD were conducted at d 37 and 45 (± 3) postpartum using a Metrichex device. The proportion of endometrial polymorphonuclear cells (PMN) was determined at 45 (± 3) DIM using a cytobrush, and cows with $\geq 5\%$ PMN were classified with endometritis (ENDO). Serum metabolites at different times were tested in mixed logistic regression models of PVD or ENDO. Predictive ability was obtained through ROC curve analysis and the optimal cut-offs with the highest Youden index were chosen. Model performance was evaluated by the area under the curve (AUC), sensitivity (Sn), and specificity (Sp). Both models included the fixed effects of parity, calving problems, and the random effect of herd. Least squares means are presented as predicted probabilities $\pm$ SE. Cows with higher Hp at 3 DIM (≥ 1.15 g/L; 17% of cows, CP in 29% of cows; AUC = 0.58, Sn = 0.37, Sp = 0.8) tended to be more likely to have PVD ($37\% \pm 9\%$ vs. $22\% \pm 2\%$, $P = 0.07$), and higher Hp at 6 DIM (≥ 0.92 g/L; 16% of cows, CP in 8% of cows; AUC = 0.61, Sn = 0.37, Sp = 0.84) were more likely to have PVD ($62\% \pm 10\%$ vs. $21\% \pm 2\%$, $P < 0.01$). Higher Hp at 3 DIM (≥ 1.24 g/L; 15% of cows, CP in 13% of cows; AUC = 0.62, Sn = 0.33, Sp = 0.88) and at 6 DIM (≥ 1.02 g/L; 16% of cows, CP in 8% of cows; AUC = 0.63, Sn = 0.36, Sp = 0.87) was associated with greater probability of ENDO [(d 3: $56\% \pm 10\%$ vs. $22\% \pm 2\%$, $P < 0.01$; d 6: $69\% \pm 11\%$ vs. $22\% \pm 2\%$, $P < 0.01$). Higher BHB at 6 DIM was associated with PVD [≥ 0.5 mmol/L (54% of cows, CP in 11% of cows); $42\% \pm 9\%$ vs. $18\% \pm 2\%$, $P = 0.02$; AUC = 0.6, Sn = 0.62, Sp = 0.55] or ENDO [≥ 1.1 mmol/L (6% of cows, CP in 25% of cows); $52\% \pm 12\%$ vs. $22\% \pm 2\%$, $P < 0.01$; AUC = 0.59, Sn = 0.3, Sp = 0.88]. Serum Ca was not associated with PVD or ENDO. These results show that excessive inflammation and hyperketonemia in the first week postpartum have a moderate ability to predict PVD and uterine inflammation near the end of the VWP.

Key Words: transition period, inflammation, endometritis

2629 Hormonally expediting first postpartum ovulation in early lactation is associated with improved uterine health in Holstein-Friesian dairy cows. T. Raz^{*1,2}, S. Druker^{2,3}, R. Sicsic², A. Kaplan³, and M. Kedmi³, ¹Advanced Academic Programs, Krieger School of Arts and Sciences, Johns Hopkins University, Baltimore, MD, ²Koret School of Veterinary Medicine, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel, ³Hachaklait, Mutual Society for Veterinary Services, Caesarea, Israel.

Uterine inflammatory diseases are prevalent in postpartum dairy cows. Studies suggest that cows spontaneously ovulate early in lactation have better ensuing reproductive performance. We aimed to evaluate the efficacy of 2 hormonal protocols (Select-sync; Select-sync-CIDR) to hastening ovulation in early lactation (treatment at 24–27 DIM) and explored the consequent incidences of cytological endometritis (CEM) and purulent vaginal discharge (PVD). Based on transrectal palpation and ultrasonography (presence of CL) combined with milk progesterone cow-side test at 24–27 DIM, postpartum Holstein-Friesian dairy cows ($n = 450$) were divided into 4 groups: Positive control: cows ovulated

spontaneously by 24–27 DIM, no treatment. Cows that did not ovulate spontaneously were allocated randomly into the following ($n = 101$ cows/group): Select–synch: GnRH analog and PGF2 α 7 d later; Select–synch–CIDR: GnRH analog and PGF2 7 d later, with 7 d CIDR; Negative control: no hormonal treatment (2 saline injections 7 d apart). Ovaries were examined by transrectal ultrasonography at 24–27, 31–34, 38–41, 45–49, and 66–69 DIM. Cytological endometritis was diagnosed by endometrial cytobrush (primiparous 38–41 DIM; multiparous 66–69 DIM), and PVD was diagnosed by Metrichex (66–69 DIM). Statistical analyses were performed in R. Time to ovulation was assessed by log-rank test; CEM and PVD incidences by logistic regression (GLM, binomial, logit). Of the 450 cows, 417 were clearly defined as ovulating or not at 24–27 DIM; 114 (27%) ovulated by the first exam, with higher rates in multiparous vs. primiparous cows (33% vs. 17%; OR = 2.44; $P < 0.05$). Both hormonal protocols efficiently increased early ovulation rates ($P < 0.05$). The Select–synch protocol was associated with a decreased risk for CEM compared with the Negative control (22.3% vs. 35.5%; OR = 0.401; $P < 0.05$). Moreover, PVD incidence was lower in Select–synch (25%, OR = 0.59; $P = 0.1049$), Select–synch–CIDR (22.3%, OR = 0.53; $P = 0.0549$), and Positive control (21.6%, OR = 0.5; $P = 0.0344$), as compared with Negative control (35.8%). We concluded that hormonal treatments can induce ovarian activity in early lactation and improve uterine health.

2630 Association of indicators of energy balance, inflammation, and stress with fecal cortisol metabolites in close-up dairy cows. C. A. Wagemann-Fluxá^{*1}, K. M. R. Lutz¹, S. J. Leblanc², E. S. Ribeiro¹, R. Palme³, and T. J. DeVries¹, ¹Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, ²Department of Population Medicine, University of Guelph, Guelph, ON, Canada, ³Department of Biological Sciences and Pathobiology, University of Veterinary Medicine, Vienna, Austria.

This study identified associations of fecal cortisol metabolite (11,17-dioxoandrostane; 11,17-DOA) concentrations with indicators of energy balance, inflammation, and heat stress, as well as hock injuries in close-up Holstein cows on Ontario commercial dairy farms. From April to October 2022, 1,498 close-up cows (primiparous [PP] = 495; multiparous [MP] = 1,003) were enrolled on 40 farms (190 $\pm$ 137 milking cows, mean $\pm$ SD). Farms were visited 6 times (1 $\times$ /4 wk), and fresh fecal samples were collected at each visit from up to 10 cows within 14 d of expected calving to measure 11,17-DOA concentrations (ng/g of fecal DM). Blood samples for measurement of NEFA (categorized as < 0.3 or ≥ 0.3 mmol/L) and haptoglobin (categorized as < 0.45 or ≥ 0.45 g/L) concentrations were collected, and maximum eye temperature (MET), hock score (1–3 scale; scores 2–3 = hock injuries), and BCS (1–5 scale; categorized as ≤ 3.25 or ≥ 3.5) were determined. Multivariable mixed-effect linear regression models were built separately for PP and MP cows to assess associations of the fixed effects of: BCS (PP = 3.7 ± 0.31 , $12\% \leq 3.25$; MP = 3.4 ± 0.40 , $45\% \leq 3.25$), NEFA (PP = 0.2 ± 0.19 mmol/L, $48\% \geq 0.3$ mmol/L; MP = 0.2 ± 0.21 mmol/L, $25\% \geq 0.3$ mmol/L), haptoglobin (PP = 0.3 ± 0.32 g/L, $9\% \geq 0.45$ g/L; MP = 0.3 ± 0.26 g/L, $8\% \geq 0.45$ g/L), MET (PP = $36.4^\circ\text{C} \pm 0.9^\circ\text{C}$; MP = $36.4^\circ\text{C} \pm 1.0^\circ\text{C}$), and hock injuries (PP = 15%; MP = 28%) with 11,17-DOA (PP = 428.0 ± 228.4 ng/g; MP = 347.0 ± 185.8 ng/g) as the dependent variable. A BCS ≤ 3.25 tended to be associated with greater 11,17-DOA in PP cows ($+54.9$ ng/g; $P = 0.06$) and was associated in MP cows ($+21.9$ ng/g; $P = 0.05$). Each 0.1-mmol/L increase in NEFA concentration was associated with greater 11,17-DOA in PP cows ($+35.1$ ng/g; $P < 0.01$), while NEFA ≥ 0.3 mmol/L was associated with greater 11,17-DOA in MP cows ($+65.7$ ng/g; $P < 0.01$). In MP cows, haptoglobin ≥ 0.45 g/L was associated with greater 11,17-DOA ($+57.1$ ng/g; $P < 0.01$). Each

0.1-unit increase in MET was associated with greater 11,17-DOA in PP cows ($+2.7$ ng/g; $P = 0.02$) and tended to be associated with greater 11,17-DOA in MP cows ($+1.2$ ng/g; $P = 0.06$). In PP cows, having hock injuries tended to be associated with greater 11,17-DOA ($+55.3$ ng/g; $P = 0.06$). The results indicate that lower BCS, hock injuries, and increased negative energy balance, inflammation, and body temperature during the close-up period are associated with a marker of stress in dairy cows.

Key Words: energy balance, inflammation, stressors

2631 The effect of midlactation hoof trimming of multiparous dairy cows on risk of subsequent lesion development. E. Shepley¹, G. Cramer^{*1}, and S. Van De Pol², ¹University of Minnesota, St. Paul, MN, ²CATTLEytics Inc., Hamilton, ON, Canada.

The objective was to determine the effect of routine hoof trimming (HT) at mid-lactation in multiparous cows with no hoof lesion history compared with no mid-lactation trim on the risk of subsequent lesion development. Second and third lactation cows from 14 dairy herds were enrolled at 117 ± 2 DIM and randomly allocated at the beginning of the week they were due to receive their mid-lactation HT to either a TRT group ($n = 9,638$), which received a mid-lactation HT, or a negative control group (CON; $n = 9,705$) that was not trimmed. Only cows in the TRT group were put on the list to receive an HT in the week of enrollment. The enrollment date was considered D 0 in our analysis for both groups. Cow demographic and hoof lesion data were extracted from a data aggregation platform (MilkShake, Cattleytics). Outcomes of interest were overall lesion prevalence risk at the next HT and time to next HT occurrence. The hazard of lesion development was analyzed using a Cox proportional hazard model that included the effect of treatment, breed composition (% Jersey), lactation, DIM at enrollment, and farm. Of the TRT cows, 6,652/9,638 (69%) were trimmed within 2 wk of enrollment. In the first 5 mo of the study follow-up, 1,845 (1,240 CON and 605 TRT) cows were trimmed. In the preliminary analysis of lesion risk, CON cows had a higher risk (4.2%) than TRT cows (2.0%). This 2.1% (95% CI: 1.5–2.5) higher risk in CON cows resulted in a 2.1 (95% CI: 1.8–2.5) higher hazard for having a lesion in CON cows. The CON cows had a 1.1% (95% CI: 0.7–1.5) higher prevalence risk of infectious lesions and a 0.9% (95% CI: 0.6–1.3) higher risk of noninfectious lesions than TRT cows. Differences varied between farms. In this study, mid-lactation HT of 2nd and 3rd lactation cows with no lesion history resulted in a slightly lower risk of hoof lesions. However, considering the slight reduction in hoof lesion prevalence, the economic benefit of a mid-lactation HT may be limited. Thus, to truly determine the benefit of HT cows with no history of lesions, the effect of HT on productivity and welfare needs to be carefully weighed.

Key Words: hoof health management, hoof trimming strategy, lameness

2632 Effect of anti-inflammatory treatment at dry-off on activity patterns during the dry and transition period in Holstein dairy cows. M. Molins^{*2}, R. Sorto¹, R. Landrum¹, L. Periconi¹, M. Marinelli¹, K. J. Harvatine², T. Ott², E. Hovingh¹, and A. A. Barragan¹, ¹Department of Veterinary and Biomedical Sciences, Penn State University, State College, PA, ²Department of Animal Sciences, Penn State University, State College, PA.

The objective of this study was to assess the effects of acetylsalicylic acid administration at dry-off on activity patterns (i.e., daily lying time, daily number of lying bouts, lying bouts duration) during the first week after dry-off and the transition period in Holstein dairy cows. On the

day of dry-off, cows were blocked by parity (primiparous [PRIM]; multiparous [MULT]) and randomly allocated to one of 2 treatments: ASA ($n = 27$): receive an oral treatment with 4 acetylsalicylic acid boluses (125 g/cow); or PLC ($n = 27$): receive an oral treatment with 4 gelatin capsules filled with water. The body condition score was measured at dry-off to group cows in BCS categories (BCSDRYCAT; optimal [OPT] = 2.75–3.5 over-conditioned [OVERC] = 3.75–5). At enrollment, an activity monitor (HOBO accelerometer) was placed on the rear leg of enrolled cows to monitor activity patterns. The data were analyzed using the UNIVARIATE and MIXED procedures of SAS as a randomized complete block design. There was a treatment, day, and parity interaction where MULT cows treated with ASA had longer lying bouts compared with PLC MULT cows the day after dry-off (ASA-MULT = 104.37 min/bout, 95% CI: 90.84–119.92; PLC-MULT = 81.09 min/bout, 95% CI: 70.96–93.17, $P = 0.02$). On the other hand, ASA OPT cows spent less time lying in the first week after dry-off compared with PLC OPT cows (ASA-OPT = 964.61 ± 30.8 min/day; PLC-OPT = $1,033.67 \pm 30.08$ min/day; $P = 0.03$). Interestingly, ASA cows tended ($P = 0.09$) to have shorter lying bouts on the day of calving (ASA = 90.53 min/bout, 95% CI: 79.39–103.20; PLC = 116.6 min/bout, 95% CI: 100.29–135.31) and the first week postpartum (ASA = 94.34 min/bout, 95% CI: 82.39–108.01; PLC = 112.71 min/bout, 95% CI: 96.05–132.28) compared with PLC cows. These results suggest that ASA treatment at dry-off may alter activity patterns during the dry and early lactation periods. Further research is needed to understand the relationship between these results and cow health and performance.

Key Words: dry-off, anti-inflammatory treatment, activity patterns

1210 Impact of pen move events on rumination time, milk yield, and water intake in dairy cows. N. Fagundo^{*}, R. Neupane, B. Shrestha, and S. Paudyal, Department of Animal Science, Texas A&M University, College Station, TX.

Pen moves within a dairy farm disrupt social dynamics of cows in the herd and affect cow behavior and performance. The objective was to evaluate the impact of pen moves on rumination time (RT; min/d), milk yield (MY; kg/d), and water intake (WI; L/d). A retrospective study was conducted on an organic dairy farm in central Texas, which consisted of 54,372 independent cow move events collected from 898 crossbred dairy cows monitored from January 2022 to June 2024. Cow-level variables including lactation stage and parity were retrieved from herd management software (Dairy Comp 305) on the farm. Daily RT and WI records were retrieved SmaXtec sensor boluses placed in the rumen of cows post-calving on their first lactation. Daily MY data were retrieved from Afimilk milk meters in the milking parlor. Statistical analysis was conducted in R using repeated measures in a mixed-effects model controlled for stage of lactation, temperature-humidity index (THI), and parity. Cow was included as random effect in the model. We identified a parity by day interaction effect on RT. Reduction in RT was more pronounced in primiparous cows on d 2 (d2), d3, d4, d5 after pen move compared with d-1 (445.57 ± 0.67 , 459.57 ± 0.67 , 455.02 ± 0.67 , 458 ± 0.68 , vs. 472.99 ± 1.84 ; $P < 0.01$). In multiparous cows, RT was only reduced on d2 and d3 post pen moves compared with d-1 (469.51 ± 0.83 , 469.41 ± 0.84 vs. 482.31 ± 0.95 ; $P < 0.01$). Similar parity-by-day interaction effect was identified in MY with a drop in milk production in d1 and d4 in primiparous cows compared with d-1 of the pen move (23.07 ± 0.08 , 22.39 ± 0.08 , vs. 22.61 ± 0.34 ; $P < 0.001$). In multiparous cows, MY was reduced on all days: d1, d2, d3 d4, and d5 compared with d-1 of pen move (21.92 ± 0.10 , 22.04 ± 0.10 , 22.21 ± 0.10 , 22.28 ± 0.10 , 22.05 ± 0.10 vs. 22.61 ± 0.34 ; $P < 0.01$). A parity by day interaction was

observed in WI, with increased WI in primiparous cows on d0, d1, and d4 compared with d-1 (80.99 ± 0.21 ; 81.15 ± 0.21 , 80.72 ± 0.21 ; $P < 0.01$). In contrast, in multiparous cows had a reduced WI from d-1 (90.30 ± 0.30) to d0 (78.05 ± 0.26), d2 (80.81 ± 0.27), and d4 (78.33 ± 0.27);

$P < 0.01$. We demonstrate that daily RT, MY, and WI are affected by pen moves, the impact of which is dependent on the parity of the cows.

Key Words: pen move, dairy cattle, cow comfort

Breeding and Genetics 3: Posters

2633 Toward standardization and completeness of milk yield recording from conventional milking systems through multistrategy optimization. A. Wang^{*1}, L. F. Brito², Y. Chang¹, W. Lou¹, and Y. Wang¹, ¹China Agricultural University, Beijing, China, ²Purdue University, West Lafayette, IN.

The rapid development of precision livestock farming highlights the critical requirement for accurate and complete large-scale phenotypic data. However, in conventional milking systems, anomalies and missing records (6.85% on average) are extremely common, which seriously impact its value on performance evaluation and farm management, such as quantifying the milk loss of cattle. This study proposed a standardized data cleaning framework for high-throughput session milk yield records from conventional milking systems by integrating and optimizing multiple strategies. The isolation forest based on deviation series with daily milk yield as the mediating variable was used for anomaly detection. For imputation of missing data, we focused on the self-imputation strategy, incorporating an adaptive averaging nearest-neighbor lactation window and the linear weighted moving average algorithm, and improving accuracy by constructing various reference populations. Based on actual patterns of anomaly and missing data, we conducted synthetic datasets and performed step-by-step validations to ensure the reliability across various scenarios. Compared with baseline strategy (SD-based method and linear interpolation), the average F1 score for anomaly detection improved by 0.13 and average R^2 for imputation of missing value improved by 0.09. Furthermore, we collected 53,598,910 session milk yield records from 44,686 Holstein cattle in China from 2019 to 2023 for validation. The results showed a substantial improvement on overall distribution and reliability of session milk yields. After data cleaning, the average R^2 of lactation curve fitting improved by 0.05, and RMSE decreased by 1.53. Additionally, this study revealed the instability of session milk yields across parities, which enhanced the informative application value of complete records and helped advance comprehension of productivity. This study emphasizes the importance of effective data cleaning strategies to ensure the completeness of session milk yield data, thereby facilitating decision-making on data-driven farms.

Key Words: session milk yield, anomaly detection, imputation of missing data

2634 Meat characteristics of purebred Wagyu steers and crosses with British and Holstein breeds. A. C. Espasandin^{*1}, R. Delpiazzo², J. Viñoly¹, M. Sanguinetti¹, and A. Rogberg³, ¹Udelar, Facultad de Agronomía, Estación Experimental Mario A. Cassinoni, Paysandú, Uruguay, ²Udelar, Facultad de Veterinaria, Paysandú, Uruguay, ³Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

The objective of this work was to characterize the meat quality of pure Wagyu-PW steers and crossbreds with British-BW and Holstein-HW, from a combined system of pasture rearing and fattening in confinement in the western coast of Uruguay. Fifty-nine animals were slaughtered, from which 2.5-cm-wide steaks were extracted from the longissimus dorsi. Luminosity (L^*) and color a^* (red-green axis) and b^* (blue-yellow axis) were determined with a Minolta CR 400 colorimeter using the CieLab scale and the meat attributes. General acceptability-AG, tenderness-T, juiciness-J, and flavor-S were studied by a consumer panel (80), on a scale of 1 to 9, with 1 being worse and 9 better, and finally indicating the preferred sample. Each consumer received one sample

per genotype of 2 cm³ with uniform cooking (70°C). The variables were subjected to ANOVA by mixed model including effects of steer genotype and the factors associated with the consumers (frequency of consumption, sex, age and the individual as random). Means were compared by adjusted Tukey's test. Genotype had significant effects for L^* , a^* and b^* ; WP was superior in L^* to WH and WB. The b^* value did not differ between PW and WH, differing with WB. Genotype was not significant for the variable a^* . Acceptability, tenderness, juiciness and flavor showed differences due to genotype, with PW and WB being superior to the WH genotype, on average with 0.5 points more on the scale. The results show similarities between WP and WB, with statistically significant differences with WH. The frequency of preference did not differ between PW and WB, (41%) being significantly lower in WH (18%). It is concluded that the attributes of Wagyu beef remain in its F1 cross with British breeds but not with the F1 cross with Holstein. Higher proportions of Wagyu in the Holstein cross could be similar in quality to WP.

Key Words: meat quality, Wagyu-Holstein cross

2635 Impact of noisy phenotypes on the accuracy of genetic evaluation. K. Das^{*1}, M. Zare¹, A. Warner¹, S. E. Aggrey², and R. Rekaya¹, ¹Department of Animal and Dairy Science, The University of Georgia, Athens, GA, ²Department of Poultry Science, The University of Georgia, Athens, GA.

Measurement accuracy in dependent variables is crucial for the reliability of research outcomes. This is particularly the case in genetic evaluation. When phenotypic data contains measurement errors, this noise can mask genetic associations, reduce classification accuracy, and could lead to biased inferences. Noisy phenotypic data are an increasingly relevant challenge due to the fast expansion in high-throughput phenotyping technologies. While modern automated and non-invasive measurement tools have enabled the collection of novel phenotypes with considerable success, a non-negligible level of measurement errors is the norm rather than the exception. In fact, several novel traits are assessed with less than 80% accuracy (e.g., greenhouse gas emissions). Even when the measurement errors are completely at random, they could bias the estimation of genetic parameters and ultimately the estimated breeding values. A simulation study was carried out to assess the impact of measurement errors in continuous phenotypes on the accuracy of genetic evaluation. A continuous trait with a heritability (h^2) of 0.1 and 0.4 was simulated, assuming a linear mixed models with one fixed effect and the additive breeding values. Measurement errors of 1%, 10%, 20%, and 30% were artificially introduced into the true simulated data to mimic accuracy of novel traits collected using automated tools. True and noisy datasets were analyzed using the same model used in the simulation. Based on 5 replicates, estimates of h^2 using noisy data decreased by 17% to 89% and 20% to 90% when the true h^2 was 0.4 and 0.1, respectively. The decrease in heritability estimates increased with the noise level. Correlation between true and estimated breeding values using the true data was 0.65 and 0.39 when the h^2 was equal to 0.4 and 0.1, respectively. Using noisy data, the correlation ranged between 0.28 and 0.62 (when $h^2 = 0.4$) and 0.15 to 0.38 (when $h^2 = 0.1$). The correlation between the true and estimated fixed effects was almost 1 independently of the noise level. Collectively, these results point toward a significant impact of even a small noise rate on the accuracy of genetic evaluation.

Key Words: noisy phenotypes, accuracy, genetic evaluation

2636 Assessing the genomic relationship of beef-on-dairy calves in Canada. L. S. F. Lopes^{*1,2}, B. O. Makanjuola², E. P. Ferdman¹, I. D. C. Hermisdorff², C. F. Baes², and D. L. Renaud¹, ¹*Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada*, ²*Department of Animal Biosciences, Ontario Agricultural College, University of Guelph, Guelph, ON, Canada*.

Beef on dairy (BOD) systems, where dairy cows are bred with beef sires, has gained popularity for improving the meat and carcass quality of non-replacement dairy calves, increasing their value. Crossbred calves also exhibit higher growth and feed efficiency rates, benefiting producers economically. However, genetic evaluation of BOD animals is challenging due to different information collection strategies, pedigree structures, allele frequencies, and the use of different genotyping panels between beef and dairy sectors, complicating parentage verification and genetic relationship assessment. Here we assessed relationships of BOD calves and their possible parents via a Genomic Relationship Matrix (GRM). Genomic data were collected from 4,992 crossbred calves genotyped with 100K GGP from NeoGen, 86 sires with a custom 72K NeoGen designed by Semex, and 117,639 cows with 29 different genotyping chips imputed to 50K by Lactanet, Canada). A total of 28,369 SNPs common to all groups was identified and a final set of 311 SNPs recommended by ICAR for parentage verification was used to build the GRM. All analyses were performed in PLINK. The average diagonal value (1.55) of the GRM for sires and calves was consistent with expectations for crossbred populations. The average genomic relationship between calves and sires was approximately 0.46 (−0.17 to 1.19). Both sires and calves had high average genomic relationship within their groups; 0.56 (0.25 to 1.62) and 0.60 (−0.15 to 2.34), respectively. Our results suggest that more common SNPs could be routinely used on beef and dairy SNP chips and highlight the need for better record-keeping and possibly more SNPs to resolve uncertain parentage cases. By reducing the number of SNPs to include only those included in the ICAR parentage reference set, a practical approach for assessing BOD calf relationships was implemented, providing a valuable tool for breeders in BOD systems to make informed selection decisions.

Key Words: beef on dairy, crossbreeding, genomics

2637 Genome-wide analysis of selection signatures in Nili Ravi buffalo. H. Mustafa^{*1}, A. Ahmad², G. Bilal³, A. Rafiq², J. Kim⁴, and S. Baloch⁵, ¹*University of Veterinary and Animal Sciences, Lahore, Pakistan*, ²*Buffalo Research Institute Pattoki, Pattoki, Pakistan*, ³*PMAS-Arid Agriculture University Rawalpindi, Rawalpindi, Pakistan*, ⁴*Yeungnam University, Gyeongsan, Gyeongbuk, Republic of Korea*, ⁵*Directorate of Animal Breeding, Sindh, Pakistan*.

Nili Ravi buffalo is a key dairy breed in South Asia, known for its superior milk production and adaptability. Identifying selection signatures can enhance genomic selection and breeding strategies. This study investigated genomic differentiation between Nili Ravi ($n = 60$) and Kundi buffalo ($n = 25$) populations using a pairwise F_{ST} analysis based on high-density SNP data (90K Axiom Buffalo SNP Chip). After quality control (call rate $> 99\%$, $MAF > 0.05$, Hardy–Weinberg Equilibrium P -value $< 1 \times 10^{-6}$), 43,389 SNPs were retained for analysis. The mean F_{ST} value across all loci was 0.682, indicating moderate-to-high genetic differentiation between the 2 populations. Twenty-four significant genomic regions ($F_{ST} > 0.65$) were identified as being under divergent selection, with the highest-ranked SNP ($F_{ST} = 0.7408$) located on chromosome 11 at position 45,506,934, near *ARPP19*, a gene involved in regulatory functions. Other notable selection signals were detected on chromosomes 1, 3, 6, 11, and 19, containing key genes such as *CSMD1*, *ROR1*, *FAM214A*, *KCNK12*, *DNAJC15*, *SVIL*, *CADMI*, *FBXW7*, *GPT2*,

and *CDH18*. These genes are associated with milk production, fertility, metabolic efficiency, stress tolerance, and immune function. A strong selection signal was observed near *ROR1* ($F_{ST} = 0.7164$) on chromosome 6, linked to multiple QTL for milk yield and reproductive performance. Additionally, *FBXW7* on chromosome 17 may play a role in metabolic efficiency and reproduction, while *DNAJC15* on chromosome 13 is associated with stress response and energy metabolism. These findings highlight genomic regions undergoing selection in Nili Ravi buffalo, providing insights for genomic selection, conservation, and sustainable genetic improvement of dairy buffalo populations.

Key Words: selection signatures, fixation index (F_{ST}) analysis, Nili Ravi buffalo

2638 Artificial intelligence for dairy calf monitoring: A comparative analysis of pose estimation models. C. S. Mussi^{*1}, A. R. Reibman¹, J. P. Boerman¹, G. C. Medeiros^{1,2}, C. A. Santos¹, I. G. M. A. Santos¹, and L. F. Brito¹, ¹*Purdue University, West Lafayette, IN*, ²*University of São Paulo, Pirassununga, SP, Brazil*.

Artificial intelligence (AI), particularly through machine learning technologies, has been increasingly applied in dairy science, offering valuable insights into new phenotypic evaluations as indicators of animal health and welfare. This study compared 2 pose estimation models by evaluating their efficiency in tracking 16 and 30 annotated keypoints on facial features of calf images, focusing on the ears, eyes, and nose. Image data from 61 Holstein calves served as basis for training the models. The YOLOv8 pose estimation model was selected for its ability to detect and analyze these key facial landmarks. The study sought to determine which model offers superior performance in monitoring calf movements, ultimately contributing to enhanced phenotyping and improved livestock management and breeding strategies. Performance was assessed using AI parameters, including training time, number of epochs, loss (error), precision, recall, and mean average precision at thresholds 0.50 (mAP50) and 0.50–0.95 (mAP50–95). The 16-keypoint model was trained faster, completing 636 epochs in 7,900 s, while the 30-keypoint model required 753 epochs in 9,667 s. However, the 30-keypoint model demonstrated lower error (2.3 vs. 3.5), suggesting improved accuracy in predicting keypoints. Precision was slightly higher for the 16-keypoint model (0.97 vs. 0.96), whereas recall was the same for both models (0.98). The mAP50 scores were nearly equivalent (0.983 vs. 0.986), but the mAP50–95 showed a difference (0.40 vs. 0.56), indicating that the 30-keypoint model was more effective in detecting visually challenging features. These findings suggest that the number of keypoints and model selection should be task-specific, considering dataset characteristics and research objectives. This study highlights the potential of AI-driven pose estimation models to enhance calf phenotyping for the future development of applications aimed at improving dairy calf management practices and breeding programs, as well as identifying stress or discomfort in calves to aid in selecting animals less susceptible to stress, which can improve animal welfare.

Key Words: dairy calf, artificial intelligence, phenotyping

2639 Developing herd-level sustainability metrics for dairy producers: A pilot study. K. L. Parker Gaddis¹, A. M. Miles², J. B. Cole^{*1,3}, and R. H. Fourdraine⁴, ¹*Council on Dairy Cattle Breeding, Bowie, MD*, ²*Animal Genomics & Improvement Laboratory, Agricultural Research Service, USDA, Beltsville, MD*, ³*Department of Animal Science, North Carolina State University, Raleigh, NC*, ⁴*Dairy Records Management Systems, Raleigh, NC*.

Sustainability is often defined by its impacts on society, economics, and the environment. This pilot study contributes to an industry-wide initiative to develop a herd-level performance metrics platform to help dairy producers track sustainability progress. The first phase focuses on 5 key areas outlined by the International Committee for Animal Recording's sustainability task force: feeding and production, fertility, health, longevity, and young stock. For each area, one trait was selected: average days in milk, average calving interval, average SCC, average culling age, and average age at first calving. It is common for different software packages or data processing centers to calculate similar traits differently, leading to inconsistencies in results. The benefit of using common definitions is that the resulting metrics can be compared over time and across data providers. Data were submitted by the 4 US dairy records processing centers (DRPC) to the Council on Dairy Cattle Breeding for analysis, totaling approximately 10,000 herds. Contemporary groups were categorized by breed, herd size (small <250, medium 251–999, large ≥1000 cows), and climate region (1–5, hottest to coldest). Extra care was taken with smaller groups to ensure adequate sample sizes while maintaining anonymity. Summary statistics and trait distributions were analyzed to identify inconsistencies across DRPC and contemporary groups. One proposed visual tool is a trait distribution for a contemporary group, with an indicator of individual herd performance, using color-coding to highlight desirable ranges when applicable. Efforts are underway to establish a routine data flow, with a current frequency of 3 times per year, aligned with the US national evaluation schedule. This system will also provide snapshots of how farm metrics change over time compared with contemporary groups. Ongoing work is focused on defining the traits most important to US dairy producers and understanding their impact on herd sustainability and resilience. With industry support, a technical plan is being developed to define a clear pathway for implementation.

Key Words: sustainability, herd-level metrics, benchmarking

2640 Trends in automated daily mobility scores, lameness, and milk yield in US commercial dairy farms. A. S. Ling^{*1}, K. L. Parker Gaddis¹, G. Cramer², and J. Burchard¹, ¹*Council on Dairy Cattle Breeding, Bowie, MD*, ²*Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN*.

Lameness has been estimated to cost the global dairy industry at least \$6 billion per year and may be a candidate trait for genetic improvement programs. Hoof lesion incidence recorded during hoof trims provides a source of data to address a common cause of lameness, but does not necessarily reflect severity or duration. Mobility scores (MS) provide a direct measure of lameness, but historically have been impractical to collect on a large scale. Recently, automated MS generated daily via overhead cameras and sophisticated machine learning algorithms have become available for use in commercial dairy operations and present a means of collecting large, dynamic lameness data. The present study compares 686,099 daily records of milk yield (MY) and MS from 6,359 cow-parities across 4 herds. Trends between MS-based lameness and daily MY were compared to determine whether MS-based lameness is associated with differences in daily MY. Mobility scores were generated by a video analytics platform and 2D camera (CattleEye, GEA) and recorded on a scale from 0 to 100 (no to extreme mobility issues). A linear regression of daily MY on herd-year, parity, and DIM class (5-d window per class) as fixed effects and MS as a covariate was conducted. The regression coefficient for MS was significant ($P < 2 \times 10^{-16}$) with an estimated effect of -0.025 kg, indicating that higher MS are associated with lower daily milk yield. When MS were collapsed into 10 classes (10-score window per class), differences between MS class effects lower than 70 were not significant, while effects for classes corresponding

to mobility 70–79, 80–89, and 90–100 were -3.09 ($P = 0.021$), -4.47 ($P = 9.31 \times 10^{-4}$), and -5.56 kg ($P = 4.77 \times 10^{-5}$), respectively. This suggests a nonlinear trend between MY and MS. Segmented regression was used to estimate a breakpoint in the regression trend at a MS of 59, with slopes of -8.3×10^{-3} and -0.11 kg estimated before and following the breakpoint, respectively. These results show that MS as measured in our study begins to have a detrimental effect on MY above a score of 59 and that scores above 70 have biologically important impacts on milk production.

Key Words: lameness, milk production, locomotion score

2641 Genetic parameters for birth weight, weaning weight, average daily gain, and serum total protein in Holstein calves. I. M. Rott^{*1}, I. J. Salfer¹, B. J. Heins^{1,2}, and I. W. Haagen¹, ¹*University of Minnesota, St. Paul, MN*, ²*West Central Research and Outreach Center, University of Minnesota, Morris, MN*.

The objective of the current study was to calculate the heritability of 4 traits in Holstein calves up to 8 weeks of age. The traits evaluated included: birth weight, weaning weight, average daily gain, and serum total protein. Calves ($n = 3,966$), from a commercial farm in southern MN, were housed at the University of Minnesota Southern Research and Outreach Center (SROC) in Waseca, MN. Calves were born between 2003 and 2023. Calves were transported to SROC twice per week and birth weight and serum total protein were recorded upon arrival. Calves were weaned at $56 (\pm 2)$ days and average daily gain was calculated from arrival to weaning. Genetic parameters were estimated with multivariate linear models with fixed effects of mean and year-month of birth for all traits. For birth weight and weaning weight, dam age was included as a fixed effect. Additive genetics was also included as a random effect for all traits. The mean birth weight for calves entering the research center was 38.7 kg ($SD = 4.34$ kg). The mean weaning weight for calves ($n = 1,693$) was 76.4 kg ($SD = 9.6$ kg). The mean average daily gain for calves from arrival to weaning was 0.67 kg/day ($SD = 0.14$ kg/day). The mean serum total protein for calves ($n = 3,958$) was 5.74 g/dL ($SD = 0.83$ g/dL). The heritability of birth weight and weaning weight were 0.69 ($SD = 0.03$) and 0.62 ($SD = 0.06$), respectively. The heritability of average daily gain and serum total protein were 0.39 ($SD = 0.06$) and 0.13 ($SD = 0.03$), respectively. For birth weight, the genetic correlations with weaning weight, average daily gain, and serum total protein were 0.79 ($SD = 0.04$), 0.49 ($SD = 0.07$), and -0.18 ($SD = 0.08$), respectively. For weaning weight, the genetic correlations with average daily and serum total protein were 0.97 ($SD = 0.06$) and -0.13 ($SD = 0.13$), respectively. For average daily gain, the genetic correlation with serum total protein was 0.47 ($SD = 0.17$). In conclusion, there is genetic variation for body and feed intake traits in Holstein calves, which can be used to make management and genetic selection decisions that result in more efficient dairy calves.

Key Words: calf efficiency, genetics, heritability

2642 Effect of coat color on fly prevalence in organic dairy heifers and cows in an experimental research herd. S. J. Fredin^{*1}, B. J. Heins^{1,2}, I. W. Haagen¹, and C. D. Dechow³, ¹*University of Minnesota, St. Paul, MN*, ²*West Central Research and Outreach Center, Morris, MN*, ³*Pennsylvania State University, University Park, PA*.

The objective of this study was to evaluate the effect of coat color on fly prevalence in grazing dairy cows and heifers during the summer grazing season. Cows and heifers were housed at the University of Minnesota West Central Research and Outreach Center, Morris, MN, and data

were collected from June 2024 to September 2024. Holsteins (n = 98) were compared with GrazeCross crossbreds (n = 127) composed of the Normande, Jersey, and Viking Red breeds and ProCross crossbreds (n = 233) composed of the Montbéliarde, Viking Red, and Holstein breeds for fly scores. Coat colors were determined from photos taken during fly scoring for all heifers and cows and were categorized as black and white (n = 52), black (n = 127), brown (n = 72), red and white (n = 16), red (n = 76), and white (n = 115). Fly prevalence was determined using a 0 to 4 scale based on fly coverage on one side of the body where 0 indicated few to no flies and 4 indicated high numbers of flies. Animals were scored once a month during the grazing season. All cattle were genotyped and imputed to 65K from Neogen (Lincoln, NE). Golden Helix, Inc. (Bozeman, MT) was used for the genome-wide association study. Dependent variables were the sum of monthly fly scores and the average of the fly scores. Independent variables for statistical analysis were coat color, cow or heifer, and herd (conventional grazing or organic). The organic herd had a higher ($P < 0.02$) sum of fly scores (5.55) compared with the grazing herd (5.02). Heifers had higher ($P < 0.001$) sum of flies (5.69) compared with cows (4.88). The average scores for the heifers (1.43) were higher ($P < 0.001$) compared with the cows (1.24). Black coat color had the highest ($P < 0.001$) average (1.57) and sum fly scores (6.23) compared with white coat color (1.20 vs. 4.67, respectively). The analysis identified 1 SNP on chromosome 17 with a strong signal. This top variant (ARS-BFGL-NGS-8026; $P = 5.21 \times 10^{-10}$) was mapped to a T > A. Results suggested that coat color may influence fly prevalence for grazing and organic cows and heifers.

Key Words: organic, fly scoring, genomics

2643 Run of homozygosity lengths in modern and lost Y-lineage Holsteins reconstituted via the USDA National Animal Germplasm Program. H. M. Kelley^{*1}, H. D. Blackburn², W. S. Liu¹, and C. D. Dechow¹, ¹*The Pennsylvania State University, University Park, PA*, ²*USDA-ARS National Animal Germplasm Program, Fort Collins, CO*.

The modern Holstein (HO) breed has only 2 Y-lineages: Chief and Elevation. Two 1960s era bulls (Cuthbert and Pilot) were reconstituted by mating modern dams with semen procured from the USDA National Animal Germplasm Program (NAGP). This study assessed lengths of runs of homozygosity (ROH) in modern and reconstituted lineages. Semen from 2 Cuthbert and 3 Pilot sons was distributed to cooperating farms to generate an outcross generation (OX) comprised of 117 heifers and 8 bulls. These were genotyped (Illumina BovineSNP777k BeadChip) along with 172 modern HO herd mates. Plink1.9 derived ROH using 758,443 SNPs after quality control with a minimum of 1Mb and 60 SNPs in length with 1 heterozygous SNP allowed. Long ROH were assumed to represent recent inbreeding whereas shorter ROH represented ancient inbreeding segments reduced by recombination. The ROH segments were grouped by length with inbreeding projected to have occurred within approximately 3 generations (G3; = 16Mb), 3 to 6 generations (G6; 8Mb–15.99Mb), 6 to 13 generations (G13; 4Mb–7.99Mb), 13 to 25 generations (G25; 2Mb–3.99Mb), and 25 to 50 generations (G50; 1Mb–1.99Mb). An ANOVA of total ROH for each generation class was conducted to determine differences by genetic lineage (modern, Cuthbert OX, Pilot OX). On average, modern HO had 515Mb total ROH which was equivalent to 19.8% of the 2.7 Gb covered by the genotyping array, Cuthbert OX had 340Mb (13.1%), and Pilot OX had 324Mb (12.4%). The 19.8% ROH coverage for modern HO was comprised of 3.94% from G3, 4.87% from G6, 4.39% from G13, 3.19% from G25, and 2.68% from G50. Cuthbert OX coverage was 1.80% for G3 and 2.56% for G50, whereas Pilot OX was 2.08% from G3 and 2.35% for G50. The modern lineage was higher ($P < 0.0001$) than Cuthbert OX and Pilot OX for G3, G6, G13, and G25. In conclusion, reintroduction of genetic lineages from the NAGP repository substantially reduced genome-wide ROH, particularly for longer segments associated with more recent inbreeding. This demonstrates the potential of use of gene banks in the restoration of genetic diversity in livestock populations.

Key Words: runs of homozygosity (ROH), gene bank, Y-lineage

Lactation Biology 3: Mammary Physiology—Posters

2644 Influence of intramammary infection and gestational stage on collagen abundance in the mammary gland of primi-gravid heifers. M. Xavier da Silva Oliveira*, K. Enger, and B. Enger, *The Ohio State University, Wooster, OH.*

Intramammary infection (IMI) in nonlactating gravid heifers can reduce future milk production. While prepartum IMI can negatively alter mammary tissue architecture, its effects on collagen deposition remain unexplored. The objective was to determine if IMI influences collagen abundance in the mammary glands of late gestation heifers. Twenty pregnant Holstein heifers at 3 gestational stage groups (6.5, 7.5, and 8.5 mo pregnant) were enrolled. Each heifer had one culture-negative mammary quarter infused with saline (SAL), while the contralateral quarter was challenged with *Staphylococcus aureus* (CHALL). Heifers were euthanized 21 d post-challenge. Mammary tissue samples were collected from 2 distinct regions: center parenchyma, immediately above the gland cistern, and edge parenchyma, adjacent to the mammary fat pad. Collagen abundance was assessed via picrosirius red staining and quantified using polarized light microscopy. Images were acquired and analyzed to determine the percentage of tissue area occupied by collagen. Data were analyzed using SAS, with the fixed effects of quarter treatment, gestational stage, mammary gland region, and their interactions. Overall, quarter treatment had no significant effect on collagen abundance ($P = 0.97$), and no interactions between quarter treatment, gestational stage, or mammary region were observed ($P > 0.37$). Collagen abundance, however, declined as gestation progressed, with 8.5-m gravid heifers having significantly less collagen than those heifers that were 6.5 m gravid ($8.14\% \text{ vs. } 11.73\% \pm 1.52\%$, respectively; $P = 0.04$). Additionally, the center parenchyma exhibited greater collagen abundance than the edge parenchyma ($11.2\% \text{ vs. } 8.8\% \pm 1.19\%$, respectively; $P < 0.01$). These findings suggest that while IMI may did not alter collagen abundance in the mammary gland of pregnant heifers, collagen abundance becomes reduced as gestation progresses and varies by mammary tissue region. Understanding these structural changes may provide insights into mammary remodeling during pregnancy and its implications for future milk production.

Key Words: mastitis, growth, pregnancy

2645 A predictive threshold value of interferon-stimulated genes in peripheral blood monocular cells for early pregnancy detection in buffaloes. R. K. Choudhary*, P. Sharma¹, S. Singh¹, N. S. Ratta², and S. Choudhary¹, ¹*College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India,* ²*Directorate of Livestock Farms, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India.*

Precise and early pregnancy detection in dairy animals is essential to improve reproduction efficiency and lactation persistency. Trophoblast cells of early concepts during the peri-implantation period release interferon-tau and sensitize the embryo for maternal recognition of pregnancy. Transcripts of the abundance of interferon-stimulated genes (ISGs) in maternal peripheral blood mononuclear cells (PBMCs) as markers of early pregnancy in buffalo remain to be investigated. We aimed to determine a predictive threshold level of 7 ISGs, namely, *ISG15*, *LGALS3BP*, *IFI44*, *RSAD2*, *OAS2*, *CLEC3B*, and *TNFSF13BP*, to determine early gestation (20/25 d post-insemination) in buffaloes. In this study, Murrah buffaloes were inseminated on d 0 (D0), and

pregnancies were diagnosed using ultrasonography (D35) and rectal palpation (D45), and blood was collected on D20, 25, and 40 for PBMC isolation. Twelve buffaloes were retrospectively classified as pregnant (P; n = 6) or non-pregnant (NP; n = 6). All 7 ISG genes were quantified using quantitative RT-PCR, and receiver operating characteristic (ROC) curves were produced to visualize diagnostic accuracies. The ROC curve for the early pregnancy diagnosis in buffalo was established based on the relative expression of individual genes at D20 and 25D compared with D0. At D20, the area under the ROC curve (AUC) of 3 genes *IFI44* > *RSAD2* > *ISG15*, were 1.0 ($P = 0.003$), 0.92 ($P = 0.01$), and 0.83 ($P = 0.01$), respectively. The cut-off value (fold change D20 vs. D0) of *IFI44*, *RSAD2*, and *ISG15* genes were greater than 3.79, 2.0, and 1.66, respectively. At D25, the AUC of the expression levels of genes of *RSAD2* > *IFI44* > *OAS2* were 1.0 ($P = 0.003$), 1.0 ($P = 0.01$), and 0.83 ($P = 0.05$ with cut off value greater than 2.1, 3.23, and 1.85, respectively. The AUC of *LGALS3BP* expression was 0.62 with a predictive cut-off value >1.36 at D20. Genes *CLEC3B* and *TNFSF13BP* were not the predictive markers of early pregnancy in buffaloes. The results of the diagnostic accuracy measurements of *ISG15*, *IFI44*, *RSAD2*, and *OAS2* in PBMC were reliable indicators of buffalo pregnancy at 20 and 25 d after insemination. The early-pregnancy-induced genes identified in this study could enable early pregnancy detection, enhancing reproductive efficiency and lactation persistency in buffaloes.

Key Words: buffalo pregnancy, interferon-stimulated genes, receiver operating characteristic (ROC)

2646 The effects of a sustained intravenous β -hydroxybutyrate infusion in combination with a systemic immune challenge at the onset of lactation. M. da Silva^{2,3}, N. Potphode³, M. Hubbard³, I. Ahn³, A. Castañeda¹, and J. E. Rico^{*1,3}, ¹*University of Pennsylvania, Kennett Square, PA,* ²*Bonn-Rhein-Sieg University of Applied Sciences, Rheinbach, Germany,* ³*University of Maryland, College Park, MD.*

Postpartum hyperketonemia (i.e., blood BHB ≥ 1.2 mmol/L; subclinical ketosis) has an erratic association with cow productivity and health. Our objective was to evaluate the metabolic, immune, and productive responses of early lactation dairy cows to sustained hyperketonemia during early lactation, before, and during endotoxemia. Eight Holstein cows (19.6 ± 5.44 d postpartum; 3.75 ± 1.9 lactations) were i.v. infused with 2.5 M solutions of either Na-BHB (n = 4; KET) or NaCl (n = 4; CON). Cows (enrolled in 2 blocks; n = 4 per block) were randomly assigned to treatments in a study with 48-h infusion periods. At 36 h of infusion, cows received an i.v. lipopolysaccharide (LPS) bolus (30 ng/kg of BW of *E. coli* O55:B5). Blood was sampled at h 0, 2, 5, 7, 10, 12, 24, 32, and 48 relative to the start of infusion, and at h 0, 1, 2, 3, 6, and 12 relative to LPS challenge. Data were analyzed under a mixed model with the fixed effects of block, treatment, time, and their interactions. We did not detect any treatment differences in DMI (43.1 ± 1.19 kg/d; $P = 0.4$), milk yield (47.2 ± 0.57 kg/d; $P = 0.15$), milk fat and protein contents ($3.93\% \pm 0.23\%$; $P = 0.88$, and $3.04\% \pm 0.11\%$; $P = 0.96$, respectively), or somatic cell count ($21.3 \pm 1.19 \times 10^3$ cells/mL; $P = 0.14$); however, milk yield was lower in KET relative to CON after the LPS challenge ($P = 0.01$). No treatment differences were detected in plasma glucose ($P = 0.38$) at any time point. Plasma NEFA differed ($P = 0.05$) between the groups throughout most time points, with KET being higher than CON. Plasma and whole blood BHB were higher in KET relative to CON ($P < 0.01$) before the LPS challenge and tended to be different after ($P = 0.06$). We did not detect any significant differences

in insulin ($P = 0.37$), TNF- α ($P = 0.51$), IL-1- β ($P = 0.91$) or IL-10 ($P = 0.31$) between the groups before or after the LPS challenge. Phagocytic activity ($P = 0.48$) and oxidative burst ($P = 0.17$) responses of neutrophils were not different between groups before endotoxemia. Our findings do not support direct negative actions of ketones on productivity or health, and further suggest that their effects may be context-dependent.

Key Words: ketosis, hyperketonemia, immune challenge

2647 Transcriptomic response of the mammary gland to air inflation. E. M. Shangraw and T. B. McFadden*, *University of Missouri, Columbia, MO.*

Our objective was to determine how the mammary gland responds to an initial elevated intramammary pressure (IMP) caused by air inflation by assessing the mammary transcriptome. We hypothesized that pathways related to milk synthesis and nutrient transport would be downregulated but pathways related to immune response, tissue remodeling, and tight junctions would be upregulated in inflated compared with control glands. Ipsilateral glands of Holstein cows ($n = 4$) were randomly assigned to 40 mmHg air inflation of both glands (TRT) immediately after a.m. milking; contralateral glands were cannulated but not inflated (CON). After treatment, glands remained inflated for 12 h, then cows were remilked. Milk samples were stripped into 50-mL tubes immediately after 0, 12, and 24 h milkings and at 4 h post-inflation. RNA was isolated from milk fat using a 2-step Trizol-column purification method. After checking RNA quality, 28 samples were sequenced: both rear quarters (1 TRT, 1 CON) for all cows at each time except at 0 h, wherein 2 cows were removed for poor RNA quality. We used DESeq2 to identify differentially expressed (DE) genes between treatments ($FDR < 0.05$, $\log_2$ fold change $> |1|$). No genes were DE at 0 h and fewer than 10 genes were DE over time in CON glands. Between treatments, there were 609, 1,226, and 1,119 DE genes at 4, 12, and 24 h, respectively. Most DE genes were upregulated, with significant increases in immune-related transcripts, particularly *CXCL8*, *OSM*, and *IL1B*. Transcripts for most milk proteins and those related to milk fat synthesis/secretion were downregulated by 12 h. However, few transcripts related to nutrient transport, tight junctions, or mechanosensing were DE. These results emphasize the link between IMP, milk stasis and involution. Combined with our physiological data for this trial, we demonstrate that raising IMP induces direct transcriptional effects on mammary secretory cells related to inflammation and lactation. Other potential factors are regulated by nontranscriptional mechanisms.

Key Words: milk stasis, transcriptomics

2648 Metabolic fingerprints of cows with low or high residual feed intake during the peripartum. A. Castañeda^{*1}, M. da Silva^{2,4}, N. Bhowmik³, C. Li³, C. P. Van Tassel³, A. M. Miles³, R. L. Baldwin³, and J. E. Rico^{1,4}, ¹*University of Pennsylvania, Kennett Square, PA*, ²*Bonn-Rhein-Sieg University of Applied Sciences, Rheinbach, Germany*, ³*Beltsville Agricultural Research Center, ARS, USDA, Beltsville, MD*, ⁴*University of Maryland, College Park, MD.*

The metabolic phenotypes of cows differing in residual feed intake (RFI) may offer cues to understanding factors driving the efficiency of nutrient utilization. Our objective was to characterize the metabolic signatures of low RFI (L-RFI; high efficiency) and high RFI (H-RFI; low efficiency) cows transitioning from gestation to lactation. Holstein cows ($n = 24$) of L-RFI ($n = 12$) and H-RFI ($n = 12$) were enrolled in a longitudinal study spanning the peripartum (21 d pre- to 21 d postpartum). Blood

and liver samples were collected and analyzed using HPLC-MS. Untargeted metabolomics data were analyzed with the multivariate platform of MetaboAnalyst 6.0. Discriminant analysis and variable importance projection scores (VIP) indicated distinct time-associated metabolic signatures in liver and blood samples between d -21 and d 7 (VIP > 1.6), while most metabolite signatures specific to L-RFI and H-RFI were observed on d 7 for liver, and on d -21 for blood (VIP > 1.8). Hepatic and blood Ala, Gly, Arg, and allantoin were higher at d 7 relative to d -21 ($FDR < 0.05$), consistent with enrichment of pathways of amino acid metabolism ($P < 0.05$). Similarly, postpartum elevations were observed in metabolites associated with enrichment of metabolism of sphingolipids, pyrimidines, glutathione, and the TCA cycle ($P < 0.05$), aligning with the adaptations of metabolism at the onset of lactation. In the liver, hexanoyl-carnitine and allantoin were higher in L-RFI cows ($P < 0.05$) on d 7, along with enrichment of metabolic pathways involved in amino acid, fatty acid, and vitamin metabolism, and with oxidative stress response ($P < 0.05$). In blood, thymine, phosphoenolpyruvate, and lactate were higher in L-RFI cows on d -21, along with enrichment of pyruvate, glycolysis/gluconeogenesis, and amino acid pathways, as well as the TCA cycle. While differences were noted between L-RFI and H-RFI cows, the most significant differences were observed between pre- and postpartum. The asynchrony in RFI differences at the prepartum in blood (d -21), and the postpartum (d 7) in liver, was evident, and may be reflective of the factors driving metabolic efficiency associated with RFI within specific metabolic compartments.

Key Words: metabolome, efficiency, biomarkers

2649 Immunoglobulin G accumulation during the prepartum period in the individual mammary glands of Holstein cows. K. Klein*, A. J. Fischer-Tlustos, J. Petrou, and J. Cant, *Department of Animal Biosciences, Guelph, ON, Canada.*

It is well documented that colostrum immunoglobulin G (IgG) concentration varies between the 4 mammary glands of a cow. However, it remains unclear if gland-specific IgG accumulation in prepartum mammary secretions indicates which quarter will have the highest IgG concentration in colostrum. The objective of this study was to assess if IgG content of quarter secretions prepartum is related to IgG content in quarter colostrum postpartum. From d -58 $\pm$ 2.9 before calving, parity 2 (P2, $n = 11$) and 3 (P3, $n = 8$) Holstein cows were dried off and fed a controlled energy diet (NEL = 1.48 Mcal/kg DM). Prepartum mammary secretions were collected by stripping a maximum of 15 mL/d from each quarter biweekly beginning at d -35 to d -14, then at d -7, and every 2 d until calving. Colostrum was collected from each gland with a quarter milker within 1.4 $\pm$ 0.76 h postpartum. Immunoglobulin G was analyzed via radial immunodiffusion. Data were evaluated using PROC GLIMMIX of SAS considering fixed effects of quarter, time and their interaction, and random effects of parity and time nested within quarter. PROC CORR was used to compare pre- and postpartum IgG concentrations for each quarter. Prepartum and colostrum IgG concentrations did not differ ($P = 0.68$) among quarters. However, quarter-averaged IgG across the udder changed over time ($P < 0.001$) with the lowest IgG concentration at d -35 (26.3 [18.5 to 37.4] g/L), greatest at d -5 (175.5 [122.2 to 252.0] g/L), and a decline to 84.9 [59.8 to 120.6] g/L in colostrum. At d -1, IgG was moderately correlated with colostrum IgG across all quarters ($r = 0.58$ to 0.75), while earlier time points were not (d -35; $r < 0.30$). Average IgG across all prepartum time points was moderately correlated with colostrum IgG ($r = 0.39$ to 0.56). These results demonstrate that quarter-specific prepartum IgG is not a good indicator of quarter-specific colostrum IgG. Timing relative to parturi-

tion plays a greater role in colostrum IgG content than quarter specific prepartum IgG levels.

Key Words: quarter-specific immunoglobulin G, colostrum, mammary secretion

2650 Oleic acid rescues saturated fatty acid-induced lipotoxicity by promoting triglyceride synthesis and restoring cellular homeostasis in bovine mammary epithelial cells. A. B. Haile* and K. J. Harvatine, *The Pennsylvania State University, University Park, PA.*

This study investigated the ability of oleic acid (OA) to rescue bovine mammary epithelial cells (MAC-T) from palmitic acid (PA)- and stearic acid (SA)-induced cytotoxicity by promoting triglyceride (TG) synthesis. We hypothesized that OA co-supplementation would protect against PA- and SA-induced lipotoxicity by facilitating TG formation and maintaining cellular homeostasis. The MAC-T cells were cultured to 80% confluence, serum-starved for 18 h, and treated for 24 h with PA, SA, and OA at 50 to 500 μ M, either alone or in combination. The experiment was conducted in triplicate, and data were analyzed by ANOVA, with means separated by a protected LSD test. Both 500 μ M PA and SA reduced cell viability observed by an MTT assay (57% and 56%, respectively; $P < 0.0001$), while co-supplementation with 250 μ M and 500 μ M of OA fully restored viability ($P > 0.0001$). Treatment with PA and SA individually downregulated the expression of TG synthesis genes (*DGAT1*, *GPAT4*, *AGPAT1*, *LPIN1*) by 60% to 80% ($P < 0.01$) at 500 μ M, but co-treatment with OA restored their expression. De novo lipogenesis genes (*ACACA*, *FASN*, *SCD*) and the lipogenic transcription factor *SREBP1C* were only partially rescued by OA. Treatment with PA quadratically increased *CD36* expression (84-fold at 250 μ M), and OA co-supplementation further enhanced expression (127-fold, $P < 0.0001$). Stearic acid also upregulated *CD36* expression to a lesser extent. These findings suggest that OA counteracts SFA-induced cytotoxicity and restores the expression of genes involved in fatty acid uptake, TG synthesis, and de novo lipogenesis, although the restoration of de novo lipogenic genes was only partial.

Key Words: cytotoxicity, triglyceride synthesis, saturated fatty acids

2651 Prostaglandin E2 decreases triacylglycerol synthesis via the prostaglandin E2 receptors EP3 and EP4 in mammary epithelial cells. C. Lalonde*¹, K. M. Lounsbury², J. Kraft¹, and F. Q. Zhao¹, ¹*Department of Animal and Veterinary Sciences, The University of Vermont, Burlington, Vermont,* ²*Department of Pathology, The University of Vermont, Burlington, Vermont.*

Prostaglandin E2 (PGE2) is a key driver of the inflammatory response and has been shown to be elevated during mastitis. There are 4 specific receptors to PGE2, namely EP1–4, all acting through different pathways. While PGE2 is important to the pathophysiology of mastitis, the effect of PGE2 on milk fat synthesis is currently unknown. The goal of this study was to investigate the impact of PGE2 on lipid synthesis in mammary epithelial cells and to determine the specific receptor(s) through which PGE2 exerts its effect, if any. Primary bovine mammary epithelial cells were cultured in DMEM/F12 media supplemented with 10% fetal bovine serum, 10 μ g/mL insulin, 10 units/mL penicillin, 10 μ g/mL streptomycin, 25 ng/mL amphotericin B, 37 mM linoleic acid, and 10 mM α -linolenic acid. Cells were seeded in 96-well plates (5,000 cells/well) and incubated 24 h in the presence of either PBS (control), 40 μ M AH6809 (a broad-spectrum prostanoicd receptor antagonist), or

one of 4 specific inhibitors for EP1–4 (250 nM GW848687X, 1.6 μ M PF-04418948, 160 nM L826.266, and 20 μ M GW627368X). Inhibitors' concentrations were calculated based on their K_i to result in inhibition >98%. All wells had a final DMSO concentration of 0.3%. Cells were then treated with 30 μ M of PGE2 and incubated for an additional 24 h. The experiment was run in triplicate. Triacylglycerol (TAG) concentration was measured from the supernatant via colorimetric assay, and cell viability was measured via MTT assays. Statistical significance was determined by one-way ANOVA using inhibitors as a fixed factor and a multiple comparison using Dunnett's method. No treatment had any significant impact on cell viability. Untreated cells produced $13\% \pm 3\%$ more TAG than cells treated with PGE2 ($P < 0.001$). Cells treated with PGE2 and EP3, EP4, or broad-spectrum inhibitors produced respectively $9\% \pm 6\%$ ($P = 0.001$), $8\% \pm 6\%$ ($P = 0.002$), and $16\% \pm 6\%$ ($P < 0.001$) more TAG than cells treated with PGE2 alone. In conclusion, our findings indicate that prostaglandin E2 reduces triacylglycerol production in bovine mammary epithelial cells, likely through at least 2 prostanoicd receptors, EP3 and EP4.

Key Words: mastitis, fat, milk

2652 Parity affects milk fat composition in primiparous and multiparous Holstein cows fed the same diet. J. K. Drackley*¹, J. A. Vasquez¹, and A. L. Lock², ¹*University of Illinois Urbana-Champaign, Urbana, IL,* ²*Michigan State University, East Lansing, MI.*

Effects of parity on milk fatty acid (FA) composition have not been studied widely. Our objective was to compare milk FA profile between primiparous and multiparous cows fed the same diet. In a randomized design, milk from 70 multiparous and 32 primiparous Holstein cows was sampled in wk 2 and wk 6 of lactation. The diet consisted of (%DM) 33% corn silage, 5.0% alfalfa silage, 5.0% alfalfa hay, 2.0% wheat straw, 3.6% cottonseed, 20% ground corn, 5.0% soy hulls, 10.5% soybean meal, 10% wet brewers grains, 1.0% blood meal, 0.9% saturated free FA, and 0.15% urea, with vitamins, minerals, and monensin. Milk fat was extracted from milk samples and methyl esters of FA were prepared by base-catalyzed transesterification. Fatty acid methyl esters were quantified by GLC. Data (milk FA, g/100 g) were analyzed using a mixed model (Proc Mixed, SAS) with repeated measures. Of the 59 FA identified, 48 were affected by parity or parity $\times$ week interaction ($P < 0.05$). The FA C16:0, *cis*-9 C18:1, *cis*-12 C18:1, C18:2 n-6, and C18:3 n-6 were greater ($P < 0.05$) or tended to be greater ($P < 0.10$) in multiparous cows than in primiparous cows; all other FA were greater ($P < 0.05$) in primiparous cows (e.g., C5:0, iso C13:0, iso C15:0, C15:0, C20:0). A total of 39 FA were affected by parity $\times$ week interaction, in most cases being different by parity at wk 2 but not wk 6. Examples include C6:0, C8:0, C10:0, C12:0, C18:0, *trans* isomers of C18:1, C18:3 n-3, CLA, and EPA. In general differences in the content of individual FA were small. As groups, the content of de novo synthesized FA and preformed FA did not differ between parities. Mixed FA content was greater ($P < 0.05$) for primiparous cows, as were total branched-chain FA ($P < 0.05$). The effect of parity might have been impacted differently by extent of body fat mobilization during early lactation, although plasma NEFA did not differ between parity groups. Differences in intake between parities might have affected ruminal metabolism of dietary FA and microbial lipid synthesis. Parity should be considered in experiments with both primiparous and multiparous cows where milk FA composition is determined.

Key Words: milk, parity, fatty acid

2653 Estimating the positional distribution of fatty acids on triglycerides from milk fat. J. K. Drackley*, *University of Illinois Urbana-Champaign, Urbana, IL.*

Determining the positional distribution of fatty acids (FA) in milk fat analytically is cumbersome. Here, I present a method to estimate FA positional distribution using only the sn-2 position FA and total FA profiles. The sn-2 FA were separated by thin-layer chromatography and sn-2 and total FA profiles of methyl-esters were determined by GLC. A series of mathematical steps resulted in mole % of FA at each position on triglycerides. First, the total yield (g/d) of each FA was determined and converted to moles per day of FA and glycerol. Next, the yield (g/d) and moles of sn-2 FA were calculated. The amounts (g/d) of sn-2 FA were subtracted from total FA yields to get sn-1,3 FA yields. Based on literature data, it was assumed that all C4 and C6 were esterified to sn-3, and that other FA would be esterified with equal probability at either sn-1 or sn-3. Accounting for the percentage of FA in the total sn-1,3 FA minus the C4 and C6 molar %, the estimated grams and molar % for sn-3 were calculated, with the remainder assumed to be at sn-1. This technique was used to estimate positional distribution of FA in milk fat from a previously published Latin square experiment with abomasal infusion of long-chain FA. Data were analyzed statistically using contrast statements to compare various treatments and positions on the triglycerides. The contrast 'sn-2 vs. sn-1 and sn-3' was significant for all FA except C18:2 (6.95, 5.84, and 4.33 mol % for sn-1, sn-2, and sn-3, respectively, across all treatments), whereas the contrast 'sn-1 vs. sn-3' was significant for all FA. Example FA data include C10, which was 6.12, 2.96, and 3.92 mol % for the control treatment and 4.52, 2.56, and 2.91 for saturated FA infusion; C16:0 (34.94, 41.95, and 22.51 for the control treatment and 34.51, 41.91, and 22.51 mol % for the saturated FA); and *cis*-9 C18:1 (18.52, 11.44, and 11.90 for the control and 23.22, 14.60, and 14.94 for the saturated FA). The molar % of FA reflected the differences in infused FA, but several FA had treatment $\times$ position interactions, which showed that different mixtures differentially affected the positional distribution.

Key Words: milk triglyceride, positional distribution, fatty acid

2654 Fatty acids at the sn-2 position of milk fat from cows abomasally infused with long-chain fatty acid mixtures. J. K. Drackley*, D. R. Bremner¹, and E. J. DePeters², *¹University of Illinois Urbana-Champaign, Urbana, IL, ²University of California, Davis, Davis, CA.*

The fatty acid (FA) esterified to the sn-2 position of milk triglycerides influences mammary triglyceride synthesis and manufacturing properties of milk fat. Our objective was to determine the sn-2 FA profile compared with the total FA profile of milk fat from cows infused abomasally with long-chain free FA mixtures. Six multiparous cows (70 DIM) with ruminal cannulas were used in a 6 $\times$ 6 Latin square design. Treatments were abomasal infusions of (1) control [168 g/d of meat solubles (carrier for FA) plus 10.6 g/d of Tween 80 (emulsifier)], (2) control plus 450 g/d of mostly saturated FA, (3) control plus 450 g/d of palm oil FA low in linoleic acid, (4) control plus 450 g/d of palm oil FA, (5) control plus 450 g/d of soybean oil FA, and (6) control plus 450 g/d of soybean oil FA with added palmitic acid. Milk fat was extracted and sn-2 FA were separated by thin-layer chromatography. Methyl esters of sn-2 and total FA were prepared and quantified by GLC. Ratios of sn-2 to total FA were analyzed using Proc Mixed in SAS to determine if the confidence limits of means included 1. The most abundant FA at sn-2 across treatments was C16:0 (mean 38.8 g/100 g), followed by *cis*-9-C18:1 (mean 17.1 g/100 g) and C14:0 (mean 17.0 g/100 g). All other detected FA

were present at <5.0 g/100 g. Treatments generally altered the content of sn-2 FA according to their FA profiles. Ratios of sn-2 FA/total FA were < 1 ($P < 0.05$) for C8, C10, C18:0, *cis*-9-C18:1, *trans*-11-C18:1, and C18:3, indicating discrimination against their esterification to sn-2. Ratios were > 1 ($P < 0.05$) for C12:0, C14:0, C15:0, C16:1, and C17:0, indicating preferential incorporation into sn-2. For C16:0, the ratios were only slightly greater ($P < 0.05$) than 1 (1.11, 1.13, 1.07, 1.01, 1.14, and 1.12 for treatments 1–6), suggesting that esterification of C16:0 was largely driven by the availability of C16:0 to mammary cells. Ratios were not greatly altered by different FA mixtures. Esterification of FA at the sn-2 position of milk triglycerides responds to availability of individual FA from the intestine but has preference for medium-chain FA from C12:0 to C17:0.

Key Words: milk fat, positional distribution, sn-2 fatty acid

2655 Milking frequency and dairy cow susceptibility to lipolysis interact to alter milk lipolysis and composition. C. Hurtaud*,¹ L. Bernard², A. Rau³, L. Rey-Cadilhac¹, and C. Cebo³, *¹PEGASE, INRAE, L'Institut Agro, Saint-Gilles, France, ²Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, Saint-Genes-Champagnelle, France, ³Université Paris-Saclay, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France.*

Milk lipolysis is defined as the hydrolysis of triglycerides resulting in the release of short-chain fatty acids responsible for rancid flavor and partial glycerides that impair functional properties. Milk lipolysis is a complex phenomenon that depends on both animal parameters and management factors. Milk spontaneous lipolysis is higher in milk from automatic milking systems, which could be due to the number and intervals of milking. In addition, considerable inter-individual variability in milk lipolysis has been observed, with some dairy cows being highly susceptible or nonsusceptible to lipolysis. The objective of this study was therefore to evaluate the impact on spontaneous milk lipolysis of different milking frequencies, that is, 1 (morning or evening), 2, or 3 milkings per day with evenly spaced milking intervals, while accounting for individual susceptibility to lipolysis. To achieve this goal, 32 dairy cows in mid-lactation were used in a continuous design with milking frequency as the main factor for a period of 3 wks. Four treatments were applied on 4 groups of cows: one milking per day at 6:00 a.m. (1M6am) or at 6:00 p.m. (1M6pm), 2 milkings per day at 6:00 a.m. and 6:00 p.m. (2M) and 3 milkings per day at 6:00 a.m., 2:00 p.m. and 10:00 p.m. (3M). In each group, there were 4 susceptible (SUS) dairy cows (lipolysis = 0.93 mEq/100 g fat) and 4 nonsusceptible (NONSUS) dairy cows (lipolysis = 0.27 mEq/100 g fat), this distribution having been made after the analysis of several milk samples per cow. Milking frequency, susceptibility to lipolysis, and their interaction were evaluated using analyses of covariance with MIXED procedure of SAS. As expected, 2M and 3M milkings increased milk yield by up to 30% compared with once-a-day milking ($P < 0.001$). We confirmed that milk spontaneous lipolysis was influenced by milking frequency: compared with 2M, we observed more lipolysis with 3M and less with 1M ($P < 0.001$). Regardless of the lipolysis susceptibility, the 1M6am and 1M6pm treatments caused a similar reduction in lipolysis. On the other hand, lipolysis was significantly higher in SUS cows with 2M and 3M treatments ($P = 0.020$). In conclusion, our results indicate increased milking frequency can adversely impact milk lipolysis. Conversely, once-a-day morning or evening milking lead significantly reduced milk lipolysis regardless of a cow's susceptibility to lipolysis.

Key Words: free fatty acid, automatic milking system, once-a-day milking

Table 1 (Abstr. 2656). Bland–Altman between manual and 3D udder measurements (n = 37)¹

Area	Observer	Bias	ULOA	LLOA	Percent within LOA
Vertical	A-3D	−0.11	4.62	−4.83	96
	B-3D	0.29	7.08	−6.49	96
	C-3D	−0.65	6.51	−7.81	96
Right quarter	A-3D	1.04	5.3	−3.22	96
	B-3D	−0.56	3.61	−4.73	96
	C-3D	0.75	5.92	−4.43	94
Left quarter	A-3D	0.75	5.4	−3.9	94
	B-3D	−0.79	3.64	−5.22	93
	C-3D	0.42	5.31	−4.48	96

¹ULOA = upper limit of agreement, LLOA = lower limit of agreement, LOA = limit of agreement.

2656 Agreement of the dairy cow udder morphometrics using manual and 3D scan measurements. J. M. Strickland*, C. I. Robison, P. H. E. Trindade, and P. L. Ruegg, *Michigan State University, East Lansing, MI.*

Changes in udder shape and size can be indicators of mammary gland conditions but can be difficult to collect. The objective of this study was to assess agreement between manually collected measurements (MM) of the hind udder and those collected with a 3D scanner (3D).

We hypothesized there would be good agreement between methods. Adult cows in tiestalls at the MSU dairy were used (n = 40). Trained observers (n = 3) measured 3 areas using a soft measuring tape, on 3 consecutive days including: vertically from the rear udder attachment to the base of the quarter and horizontally on each quarter from medial cleft to hind leg juncture. Marks were placed on the udders to delineate MM areas. One observer collected 3D scans (Structure Sensor, Occipital, CA) and measured using 3-Matic Software (Materialise, Belgium). Cows with mastitis were removed (n = 3). Statistics were done with R Studio (Boston, MA). Intraclass correlation (ICC) among observers and between MM and 3D were analyzed. Bland–Altman was used to assess bias between MM and 3D. Linear regression was used to assess proportional bias. The inter-observer reliability was good to excellent for MM (ICC ≥ 0.88, *P* < 0.001). Reliability between MM and 3D was good to excellent for all observers (ICC ≥ 0.87, *P* < 0.001). Bland–Altman assessment found minimal bias between MM and 3D, except for observer A right quarter (Table 1). Observers A and C consistently underestimated the vertical and overestimated the quarter MM compared with 3D. Also, 93%–96% of results were within the upper and lower bounds of agreement. The linear regressions found that observer C had positive proportional bias for vertical and right quarter measurements, meaning as udder size increased, MM were overestimated compared with 3D (*P* = 0.03). These results indicate that there is satisfactory agreement between the 2 methods.

Key Words: udder morphometrics, 3D scanner

Physiology and Endocrinology 3: Posters

2657 Peripheral blood mononuclear cell mitochondrial enzyme activity associations with embryo transfer success in dairy cattle recipients. A. G. Waymire* and H. A. Rossow, *Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California, Davis, Tulare, CA.*

Mitochondria produce energy vital for health, reproduction, and metabolism and have their own maternally inherited DNA. The objective of this study was to identify if higher mitochondrial enzyme activities (MEA) of Complex I (CI), Complex IV (CIV), and Complex V (CV) in peripheral blood mononuclear cells (PBMC) were associated with higher success rates of embryo transfer (ET) in recipients. Whole blood was collected from 40 Holstein cows via coccygeal venipuncture from June to September 2024. Recipients were assigned either embryo A (Donor 1/Sire 1, n = 20) or embryo B (Donor 2/Sire 2, n = 20) based on donor embryo production and recipient availability. Crude mitochondrial extracts were isolated from PBMC and analyzed for MEA of CI, CIV, and CV (Abcam, Cambridge, UK). Pregnancy status was checked at approximately 32 d carried calf (DCC) and confirmed at 45 and 90 DCC. Management, genomic, and ET outcome data were collected from DC305 (VAS, Tulare, CA). Enzyme activity was regressed on the independent variables pregnancy status, embryo, DIM at enrollment (DIMIT), MEA (CI, CIV, CV), red blood cell (RBC) count, hemoglobin (HB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), age at first calving (AGEFC), weight at first calving (WGTF), wk 4 and 8 daily milk yield, and genomic daughter pregnancy rate using the GLM procedure of SAS (SAS 9.4, SAS Institute Inc., Cary, NC). Hematological variables were included considering PBMC are immune cells, in addition to management and genomic variables that can influence reproductive success. The activities of CI, CIV, and CV were significantly associated with RBC, MCHC, DIMIT, AGEFC, and WGTF, in addition to HB and MCH solely for CIV and CV. This aligns with hematological roles in oxygen transport, which sustains cellular respiration and energy production. Associations with AGEFC, WGTF, and DIMIT showcase metabolic state influences MEA. Complex I, IV, and V activities were not associated with success or failure of pregnancy up to 90 DCC. Associations between recipient MEA and live birth or ET calf survivability remain to be determined.

Key Words: mitochondria

2658 In utero heat stress affects the ovarian proteome of dairy calves at weaning. Y. Butenko¹, B. D. Davidson², G. E. Dahl³, J. Laporta², and M. Zachut^{*1}, ¹*Department of Ruminant Sciences, Institute of Animal Sciences, Agriculture research Organization, Volcani Institute, Rishon LeZion, Israel,* ²*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* ³*Department of Animal Science, University of Florida, Gainesville, FL.*

Prenatal hyperthermia during late gestation in dairy cows affects a myriad of offspring phenotypes, including reproductive-related parameters. Here, we examined the effects of in utero heat stress on the ovarian proteome of dairy heifers. Multiparous dams were heat stressed (HT; shade) or cooled (CL; shade, fans, water soakers) during the last 56 d of pregnancy and gave birth to in-utero heat stressed (IUHT) or in-utero cooled (IUCL) calves, respectively. A subset of calves was euthanized at 1 week after weaning to harvest ovaries (n = 8 calves/treatment). Protein lysates were extracted for proteomic analysis of IUHT and IUCL ovaries (n = 5 calves/treatment) using LC-MS/MS; one sample from

each group was an outlier and excluded from further analysis. Bioinformatic analysis (n = 4 calves/treatment) was conducted using *t*-tests [$P \leq 0.05$ and fold change (FC) ± 1.2] and pathway analysis (Ingenuity software, Qiagen). A total of 8,327 proteins were identified, and 145 proteins showed differential abundance between IUCL and IUHT. Proteins related to ovarian function were differentially abundant between groups, notably, the abundance of pyruvate dehydrogenase kinase 4 (PDK4; $P = 0.03$; FC = 1.80) and cadherin 1 (CDH1; $P = 0.04$; FC = 1.23) were higher in IUCL vs. IUHT. Upregulation of PDK4 delays the aging process of postovulatory oocytes and improves mitochondrial activity, whereas CDH1 is involved in establishing the germ cell lineage, oocyte growth, and meiotic competence during gonad development in mice. In addition, the abundance of aryl hydrocarbon receptor (AHR; $P = 0.04$; FC = -1.83) and arginase 1 (ARG1; $P = 0.003$; FC = -6.01) were lower in IUCL vs. IUHT ovaries. Both AHR and ARG1 play roles in cellular responses to oxidative stress, with ARG1 acting indirectly by modulating nitric oxide availability. Their upregulation in the IUHT ovaries may lead to increased production of reactive oxygen species (ROS). Several biological functions related to ovarian morphology and development were enriched based on differential proteins in IUCL vs. IUHT ovaries. These data portray the vast ovarian proteome of dairy calves, highlighting proteins related to ovarian function and oxidative stress that were affected by IUHT. Overall, this data contributes to our understanding of the effects of IUHT on ovarian function in dairy calves.

Key Words: in utero heat stress, ovary

2659 THI breakpoints for rectal temperature and milk yield in lactating Cinisara cows under a semi-extensive system. C. Cavallo¹, M. Barbato¹, C. Evola¹, InnovaRe Consortium², L. Liotta¹, and V. Lopreiato^{*1}, ¹*Department of Veterinary Sciences, Università degli Studi di Messina, Messina, Italy,* ²*Innovare Consortium, Napoli, Italy.*

In livestock, thermoregulation is a critical aspect of adaptation to harsh environments and low-management breeding systems, which are often features of local breeds. Cinisara is a native cattle breed from Sicily, the southernmost region of Italy, with a black coat and primarily used for dairy production. This study evaluated the regulation of body temperature and milk response of Cinisara cows under heat stress. A total of 35 lactating Cinisara cows [Days in milk (DIM): 144 ± 52 d; milk yield (MY): 20 ± 2.4 kg/d; parity: 1.5 ± 0.6] were monitored between June and September. Cows were reared in a semi-extensive system. Temperature-humidity index (THI) was recorded every 15 min by 3 thermo-hygrometers installed in the feeding area, in a shady and sunny area at grazing, and rectal temperature (RT) was measured monthly at 0700 h and 1600 h. Milk yield was recorded and milk analyses were performed. PROC GLIMMIX of SAS was used with the fixed effect of time (June, July, August, September) for milk, and of time, hour of day (0700 h, 1700 h), and their interaction for RT. PROC NLIN was also used. The highest RT was recorded in August, coinciding with a daily mean THI of 78 (38.5°C and 39.2°C at 0700 h and 1600 h, respectively). Between THI of 65 and 83 (morning and afternoon THI), Cinisara cows had a THI breakpoint for the RT of 77, with RT increasing by 0.05°C for each unit increase in THI below the threshold, and by 0.12°C above it (R^2 adj. = 0.90). The MY recorded was 20.1, 17.5, 13.4, and 18.3 kg/d at mean THI of 73, 77, 78, and 76, respectively, showing a linear decrease up to THI 76, followed by a drop, with the lowest MY at THI 77 ($P < 0.05$). The THI breakpoint for MY was 76 (R^2 adj. = 0.87). Milk fat, protein, and lactose content were lowest at the highest mean THI,

showing a linear decline with increasing THI. Milk urea content was lowest at THI 77, suggesting that urea metabolism was more influenced by pasture availability and intake than muscle catabolism. This study established the THI breakpoints for RT and MY in Cinisara cows, highlighting improved thermoregulation and adaptation in terms of MY during heat stress, despite the low-management conditions that lack cooling systems or alternative heat-stress mitigation strategies. Research granted by Agritech CUP: J83C22000830005-Innovare.

Key Words: heat stress, THI threshold, Cinisara

2660 Plasma lipidomic analysis reveals biomarkers and metabolic pathways associated with heat-stress resistance in lactating Holstein dairy cows. O. A. Odunfa^{*1}, T. N. Marins², S. Tao², and Y. Jiang¹, ¹University of Wisconsin–Madison, Madison, WI, ²University of Georgia, Athens, GA.

This study aimed to identify plasma lipid biomarkers and pathways associated with heat-stress resistance (HR) in dairy cows. Sixteen multiparous lactating Holstein dairy cows were enrolled and fed TMR once daily. The study included a 10-d baseline and 49-d heat stress phase (deprivation of evaporative cooling). Vaginal temperature (VT) was recorded every 5 min from d -1 to 3. The 4 cows with the smallest increase (1.4%–2.7%) in mean VT from d 1 to d 3 of heat stress, relative to baseline served as HR, while the remaining 12 cows served as controls. Blood samples were collected on d -3 (baseline) and d 1 (heat stress) before feeding. Lipidomic analysis of plasma was performed using liquid chromatography-mass spectrophotometry, and data were analyzed using MetaboAnalyst 6.0. Pathway analysis was done using Bioinformatics Methodology for Pathway Analysis (BioPAN). On d 1 of heat stress, concentrations of 46 plasma lipids were increased and concentrations of 83 lipids were decreased in HR cows. The most active pathway was DG (diacylglycerol)→PE (phosphatidylethanolamine) while PE (O-16:1_20:3)→ phosphatidylcholine, PC (O-16:1_20:3) was the most suppressed pathway in HR cows. Before heat stress (d -3), the most active pathway in HR cows was DG→PC, while the most suppressed pathways were PC(O-32:0)→ phosphatidylserine PS(O-32:0) and PC(O-31:0)→PS(O-31:0). On d -3, HR cows had higher concentrations of 45 plasma lipids and lower concentrations of 48 lipids compared with the control. The most promising biomarkers for predicting heat stress resistance in dairy cows were PI-Cer (phosphatidylinositol ceramide) 40:4;O3, DG 44:8, SM (sphingomyelin) 41:2;O2, and HexCer (hexosylceramide) 18:0;O2/24:1;O (area under the curve [AUC] values >0.98). Our results indicate that, even before heat stress, HR cows exhibit differential abundances of lipids and lipidomic pathways. Further studies should validate the identified biomarkers for future selection of HR dairy cows.

Key Words: heat resistance, lactating cow, plasma lipidome

2661 Extrahepatic glucose metabolism, but not hepatic gluconeogenic or oxidative gene expression or mitochondrial oxidative capacity, may differ by RFI status. S. J. Kendall¹, T. L. France¹, M. Paiva Rodrigues¹, S. J. Johnson¹, U. Arshad¹, M. J. VandeHaar², Z. Zhou², F. Peñagaricano¹, K. A. Weigel¹, and H. M. White^{*1}, ¹University of Wisconsin–Madison, Madison, WI, ²Michigan State University, East Lansing, MI.

Investigation into molecular pathways to determine if there are innate differences driving feed efficiency (FE) has not been fully scrutinized. The objective of this study was to determine if (1) mitochondrial function via oxygen consumption rate (OCR; pmol O₂/min/μg protein), (2)

expression of genes involved in uncoupling, the TCA cycle, and glucose metabolism, and (3) tail vessel and mammary vein metabolites differ by FE status. Multiparous mid-lactation Holstein cows (n = 64) were enrolled on an 8-wk FE study. Residual feed intake (RFI) was calculated in wk 6. In wk 8, liver tissue and blood samples were collected from the top and bottom 20% of cows based on wk 6 RFI. Post-study, RFI was recalculated, and the most divergent RFI cows were identified and chosen for analysis (n = 5 +RFI (lowFE); n = 5 -RFI (highFE)). All data were analyzed (PROC MIXED, SAS, 9.4) with fixed effect of RFI status. Metabolite and gene expression data included random effect of sampling day; mitochondrial function data included additional fixed effects of time, RFI × time, and random effect of plate and cow nested within RFI status. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. Expression of hepatic genes associated with uncoupling, TCA cycle, and glucose metabolism did not differ between +RFI and -RFI cows ($P \geq 0.19$). Mitochondrial function was measured for complex I and IV, and for complex II and IV, via OCR. No differences in +RFI and -RFI OCR nor RFI × time were detected ($P \geq 0.62$), although OCR fluctuated over time ($P < 0.0001$) as expected due to assay design. Tail vessel metabolites, including ALT, AST, BHB, BUN, cholesterol, glucose, insulin, NEFA, and triglycerides, did not differ by RFI status ($P \geq 0.23$). Mammary vein glucose was greater in -RFI cows than +RFI cows ($P = 0.01$; 52.96 vs. 42.31 ± 2.72 mg/dL), and mammary vein insulin was greater in -RFI cows than +RFI cows ($P = 0.002$; 0.76 vs. 0.21 ± 0.20 μg/L). Taken together with past studies, these results suggest that extrahepatic glucose metabolism may be a source of variance between high vs. low RFI cows.

Key Words: glucose metabolism, feed efficiency, mitochondrial function

2662 Assessment of glutathione peroxidase activity in AML12 cells treated with different concentrations of GSH following oxidative stress stimulation. A. F. Lima^{*} and J. S. Osorio, School of Animal Science, Dairy Science, Virginia Tech, Blacksburg, VA.

Oxidative stress occurs when there is an imbalance between increased oxidative factors, such as reactive oxygen metabolites, and depletion of antioxidant capacity. Glutathione peroxidase (GSH-Px) is an essential antioxidant mechanism that contributes to the reduction of hydroperoxides by utilizing glutathione (GSH) as a reducing agent. The objective of this in vitro study was to investigate whether an increased supply of GSH would enhance the GPx activity in the α mouse liver 12 (AML12) cell line. Cells were harvested at 85% confluency by trypsinization, and after counting, cells were transferred to 6-well plates, 7×10^5 cells/well. The cells were incubated for 24 h at 37°C and 5% CO₂ in a DMEM/F12 medium. After 24 h (70%–80% confluency), the cells were cultured in a medium containing 3 different concentrations of GSH for another 24 h, followed by 6 h incubation with 100 μM hydrogen peroxide (H₂O₂), which was used to stimulate oxidative stress in vitro. Treatments were control (GSH1; culture medium without any treatment), 1.2 nmol of GSH (GSH2), 2.4 nmol of GSH (GSH3), and 4.8 nmol of GSH (GSH4). Cell viability and GSH-Px activity were measured. Additional cells were stored in trizol at -80°C until RT-qPCR analysis of genes related to GSH metabolism. Data were analyzed using the MIXED procedure of SAS, including 5 batch replicates run on different days. Significance was declared at $P \leq 0.05$ and trend at $P \leq 0.10$. A trend ($P = 0.06$) was observed for GSH treatment effect on GSH-Px activity, derived from an increased GSH-Px activity in GSH1 compared with GSH4 treatment following H₂O₂ exposure (26.6 vs. 20.0 mU/mL). Additionally, a linear decrease ($P < 0.01$) in GSH-Px activity was observed with increasing GSH concentrations. However, no significant differences ($P \geq 0.18$)

were found in cell viability or total count. Contrary to expectations, increased supply of GSH did not enhance GPx activity but instead diminished it. These findings suggest that the role of GSH in oxidative stress regulation may be more complex than anticipated, warranting further analysis from this project.

Key Words: glutathione, oxidative stress, AML12

2663 Autophagy modulation by dihydromyricetin alleviates oxidative stress in mammary epithelial cells of dairy cows. S. J. Guo, Z. Z. Liu, H. Zhang, Z. X. Zhao, and J. J. Tong*, *Beijing University of Agriculture, Beijing, China.*

Oxidative stress disrupts mammary gland immunity in dairy cows, leading to epithelial inflammation, apoptosis, and reduced milk production. Autophagy helps mitigate oxidative damage by maintaining cellular homeostasis. Dihydromyricetin (DHM), a flavonoid with antioxidant and anti-inflammatory properties, modulates mitochondrial autophagy, but its protective role against oxidative stress in mammary epithelial cells remains unclear. This study aimed to investigate the protective effects of DHM against oxidative stress in mammary epithelial cells (MAC-T) and to elucidate its role in mitochondrial autophagy regula-

tion. The MAC-T cells were divided into 4 groups ($n = 6$): control, DHM ($8 \mu M$), H_2O_2 ($800 \mu M$), and DHM + H_2O_2 . Cell viability, apoptosis, mitochondrial ROS, and membrane potential were evaluated via CCK-8, Annexin V/PI staining, MitoSOX Red, and JC-1 staining. Proteomic analysis identified differentially expressed proteins and pathways, while autophagy-related gene and protein expression were assessed using RT-qPCR and western blot. Statistical significance was determined by one-way ANOVA with Tukey's post hoc test ($P < 0.05$). The DHM treatment significantly improved cell viability and reduced apoptosis ($P < 0.05$) while lowering mitochondrial ROS levels in the DHM + H_2O_2 group ($P < 0.05$). Proteomic analysis revealed 325 upregulated and 704 downregulated proteins in the DHM group, with enrichment in autophagy-related pathways, particularly the lysosomal pathway. The RT-qPCR and western blot confirmed that DHM upregulated *PINK1*, *Parkin*, *BNIP3*, *LC3*, and *Beclin-1* while downregulating *P62* ($P < 0.05$), indicating enhanced mitochondrial autophagy. Therefore, DHM enhances mitochondrial autophagy and reduces apoptosis in mammary epithelial cells under oxidative stress, highlighting its potential to improve mammary health.

Key Words: dihydromyricetin, oxidative stress, mitochondrial autophagy

Production, Management, and the Environment 3: Posters

2664 Investigating dairy cattle welfare on Iranian intensive farms using resource-based measures: Feed and water availability. A. Jafari-Gh^{*1}, R. Laven¹, S. M. H. Mirrahimi², S. Jafari-Gh³, F. Khaloubaghari⁴, K. R. Mueller¹, M. Dehghan Banadaky⁵, and E. Vallee¹, ¹*School of Veterinary Science, Massey University, Palmerston North, New Zealand*, ²*School of Veterinary Medicine, Karaj Islamic Azad University, Karaj, Iran*, ³*Animal Science Department, College of Agriculture and Natural Resources, Karaj Islamic Azad University, Karaj, Iran*, ⁴*Independent Researcher, Palmerston North, New Zealand*, ⁵*Animal Science Department, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran*.

Freedom from thirst and hunger is the first of the 5 freedoms. Despite this, a 2021 review by Jensen and colleagues showed that cows are deprived of drinking water on many farms worldwide, although the same has not been reported for feed. We designed and used a welfare assessment protocol that included resource-based measures such as those related to availability of and access to clean feed and water. The protocol was used on 28 freestall (FS) and 34 bedded-pack (BP) farms in 2022 to 2023. The BCS was visually scored using the 1 to 5 system. Resource-based measurements were done when cows were out for milking. Descriptive statistics were calculated using SAS version 9.4. Results show that the median (IQR) feed space per head of fresh (DIM <21) and early lactation (DIM 21–150) cows in FS farms were 114 cm (70–143) and 66 cm (56–80), respectively. The same figures for cows in BP farms were 83 cm (42–102) and 63 cm (44–71), respectively. Additionally, median (IQR) water trough space per head of fresh and early lactation cow in FS farms were 14 cm (7.5–18) and 7.5 cm (5.8–13), respectively, whereas the same figures for cows in BP farms were 7.1 cm (3.4–17) and 7.3 (3.8–10), respectively. Three FS and 2 BP farms provided access to water at parlor exit, but the median (IQR) of distance to water trough after parlor was 55 m (30–120) in FS farms and 48 m (28–70) in BP farms. Even though the median water trough space/cow meets the standards (i.e., minimum of 6 cm/cow), there were 28% of farms with insufficient water trough space, with 10% having <3 cm of space per cow, and 13% with at least 1 pen being deprived from water at the time of visit. This is important because the study farms were in arid and semiarid areas with high chances of heat stress. Water cleanliness was another welfare issue, as only 35% of farms provided their cows with clean water in clean troughs. Similarly, feed trough space per cow was lower than the standards (i.e., minimum of 47 cm/cow) in 20% of farms and was dirty in 15% of farms. Despite this, median (IQR) of herd-level BCS in cows with DIM ≤150 was 2.75 (2.75–2.88). More research is needed to investigate the relationship between feed and water trough space and cleanliness with BCS.

Key Words: cattle welfare, cattle nutrition, farm management

2665 Effect of calf age at castration on health and performance in young dairy origin calves. D. Langenkamp^{*} and J. Gawthrop, *CalfCare, North Manchester, IN*.

Castration is a common procedure in cattle for reproductive control and safety of both man and animal. It can also be a painful and stressful procedure. The current literature would suggest castrating earlier in life can help mitigate stress and pain associated with castration. These current data are based on castrating closer to 3 mo of age. The objective of this study was to measure the health and performance impacts of castrating at different ages in young dairy origin calves. One hundred

thirty cross-bred calves 3 to 7 d of age were used in a randomized clinical trial. Calves were sourced from a sale barn and shipped to a commercial raising facility. Heifer calves were used as a negative control (CON, n = 59). Bull calves were randomly assigned 1 of 3 treatments: castration upon arrival (C0, n = 24), castration at 21 d of age (C21, n = 24), or castration upon weaning at 42 d of age (CW, n = 24). The C0 calves were administered meloxicam at the time of castration on d 0. The C21 and CW calves were administered meloxicam the night before the procedure. Calves were castrated using castration shears by cutting the scrotum and pulling out the testicles. The castration site was sprayed with Durvet Screw Worm Aerosol spray after the procedure. Upon enrollment on d 0, calves were weighed and blood was drawn to measure total serum protein (TSP) levels. Calves were weighed on d 0, 7, 28, 49, and 66. Least squares means were analyzed using the Microsoft Excel Data Analysis Toolpak (Microsoft, Redmond, WA). There was no difference in TSP levels upon arrival ($P = 0.38$). The CON calves were lighter upon arrival ($P = 0.002$), on d 7 ($P = 0.003$), d 28 ($P = 0.01$), and d 49 ($P = 0.03$). There was no difference in weight gain at d 7 ($P = 0.45$), d 28 ($P = 0.78$), d 49 ($P = 0.57$), or d 66 ($P = 0.87$). Although not statistically significant, C0 calves had lower average total treatment costs per head ($P = 0.25$): C0 = \$0.41, C21 = \$2.26, CW = \$3.47, CON = \$1.63. There was no mortality for any of the treatment groups. Further investigation with a larger population size is needed to determine if age at castration affects the health and performance of young dairy origin calves.

Key Words: castration, calf

2666 Holstein sale barn calves evaluated for total serum protein concentrations. D. P. Casper^{*1,2} and T. Liu³, ¹*Casper's Calf Ranch, LLC, Freeport, IL*, ²*North Carolina A&T University, Greensboro, NC*, ³*College of Animal Science and Technology, Gansu Agricultural University, Lanzhou, China*.

Calves are born without immunity. Colostrum consumption by the calf is critical for transferring immunity from the mother to the calf. Male dairy calves are routinely sold off the dairy operation to calf raisers and feedlots for meat production. Recently, recommendations were published for female dairy calves, but no recommendations were given for male dairy calves. A simple method for assessing the transfer of passive immunity is measuring total serum proteins (TSP) within the first days of life. Total serum proteins are routinely measured in our contract research operation studying neonatal calf nutrition. The TSP concentrations for 1,107 male Holstein calves from the past 5 yr across 16 separate experiments were measured using an optical or digital refractometer. The mean TSP concentration was 5.36 g/dL with a range from 2.8 to 8.0 g/dL with a CV of 14.1% and SD of 0.77. Mean TSP concentrations increased with year (4.94, 5.40, 5.37, 5.41, 5.58 g/dL for 2020, 2021, 2022, 2023, and 2024, respectively), indicating that dairy producers are improving their colostrum program for newborn male Holstein calves. The latest heifer immunity transfer status recommendations are excellent (>40%), good (>30%), fair (>20%), and poor (>10%), but these data percentages for male Holstein calves were, 14.4%, 13.2%, 37.0%, and 35.5%. Thus, a large percentage of the calves are in the poor and fair categories. However, the percentage of poor TSP status has declined with time (60.0%, 33.3%, 33.2%, 30.1%, and 27.7%, for 2020, 2021, 2022, 2023, and 2024). Death loss was 3.6% among these experiments and years. The death loss within categories was 5.0%, 15.0%, 37.5%, and 42.5% for excellent, good, fair, and poor. In conclusion, dairy opera-

tions are improving the colostrum management of male Holstein calves to minimize failure of passive immunity, but further improvements are possible to achieve heifer immunity status standards.

Key Words: calf, colostrum, total serum protein

2667 Validation of a positive pressure tube ventilation system for heat abatement of preweaned dairy heifers. M. C. Guenther^{*1}, B. D. Davidson¹, C. G. Savegnago², C. E. Halbach¹, S. Tao², and J. Laporta¹, ¹University of Wisconsin–Madison, Madison, WI, ²University of Georgia, Athens, GA.

Dairy calves are often overlooked for heat abatement strategies. Therefore, we sought to design and examine the effectiveness of a positive pressure tube ventilation system (PPTVS) for preweaned heifers housed outdoors during a subtropical summer (temperature-humidity index, THI >79). Two-wk-old Holstein heifers were individually housed in wire hutches under a shade cloth structure with a PPTVS to supply consistent airflow with daytime misting for cooling treatment (CL, n = 12). Airspeed was recorded at 0.3, 0.9, and 1.5 m from the PPTVS outlets. Calf respiratory rate (RR), skin temperature (ST), and rectal temperature (RT) were recorded at 3 locations (LOC; near, far, or blocked) after a 45-min restriction at each LOC. In addition, airspeed, RR, ST, and RT were recorded from adjacent heifers without the PPTVS (HS, n = 6) to examine similarities between blocked and HS. All data were collected every 6 h at 6 time points within a continuous 30-h period. Hutch THI was measured continuously and averaged per hour. Data were analyzed using mixed models. Airspeeds measured 0.3 m from the PPTVS were faster than at 0.9 and 1.5 m (3.0, 1.5, 1.0 m/s, respectively; $P < 0.01$), all of which were faster than the HS hutch (0.31 m/s; $P < 0.01$). The CL hutch microenvironment had a lower THI than HS hutches (81.4 vs. 83.2; $P < 0.01$), and this improvement was most pronounced during night-time hours (Trt $\times$ Hr; $P < 0.01$). Heifer RR, ST, and RT were similar between HS and blocked LOC ($P > 0.10$). Heifers restricted near the PPTVS outlet had lower RR than far and blocked (37.7, 45.5, 50.3 breaths per minute, respectively; $P < 0.05$). Even during early afternoon hours, the RR was decreased in near compared with blocked (LOC $\times$ Time point; $P < 0.01$). Heifers restricted near and far had lower ST than blocked (32.5, 32.9, 33.7°C, respectively; $P < 0.05$). This change was most pronounced during nighttime hours (LOC $\times$ Time point; $P < 0.01$). The LOC within a hutch did not affect RT ($P = 0.85$). Providing a PPTVS with daytime misting improved hutch microclimate and improved thermoregulatory parameters of preweaned heifers exposed to heat stress.

Key Words: microenvironment, thermoregulation, heat stress

2668 Construction of a comprehensive heat stress index for dairy cows. N. Cao^{1,2}, P. Lemal², L. Ma¹, N. Gengler², and D. P. Bu^{*1}, ¹State Key Laboratory of Animal Nutrition and Feeding, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, ²Gembloux Agro-Bio Tech (ULiège-GxABT), University of Liège, Gembloux, Belgium.

Heat stress significantly reduces dairy cow performance, negatively impacting the dairy industry's economic efficiency. Accurate monitoring of heat stress responses in dairy cows is crucial for effectively preventing and managing heat stress conditions. Currently, the ambient temperature and humidity index (THI) is commonly used to assess the degree of heat stress in dairy cows. Although it provides a useful general indication of heat stress at the population level, it does not account for individual differences between cows. To more accurately assess the degree of heat stress in dairy cows, this study integrated multiple indicators, includ-

ing behaviors and milk traits, to construct a new and comprehensive evaluation index. In this study, 1,027 Holstein cows were selected under THI >78, including 455 primiparous cows and 572 cows in their second and third lactations. Data on activity, lying time, respiration rate, rectal temperature, salivation score, daily milk yield, milk fat, milk protein, milk lactose, SCC in milk, BCS were recorded. To eliminate the effects of parity and days in lactation on milk production, residual values of milk yield were calculated using a general linear model. The residuals of milk yield were used as the dependent variable. The model was built using principal component regression, ridge regression, LASSO regression, and elasticity regression. The results showed that the LASSO regression model performed best, with a lambda value of 0.01 and an R^2 of 0.25. Finally, the parameters were normalized using a standard scoring function, and the weighted and normalized phenotypic parameters were integrated into a comprehensive heat stress index (MYT). The results showed that lying time, BCS, and rectal temperature contributed the most weight to the MYT index, which were 0.34, 0.16, and 0.16, respectively. The MYT index determined a threshold of 0.49 to assess whether a cow is experiencing heat stress. The correlation coefficient between the MYT index and the residual value of milk yield was -0.46 , indicating that milk production decreases as the residual value increases, and the MYT index decreases accordingly. The MYT index provides a new tool for personalized heat stress assessment, improving management and dairy cow performance.

Key Words: heat stress, residual, comprehensive index

2669 Characterization of extracellular vesicles at parturition in dairy cows with late gestation heat stress. L. T. Casarotto^{*1}, L. Galio², C. Henry³, P. Chavatte-Palmer², and G. E. Dahl¹, ¹University of Florida, Gainesville, FL, ²Université Paris-Saclay, UVSQ, INRAE, BREED, Jouy-en-Josas, France, ³Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, PAPPISO, Jouy-en-Josas, France.

Extracellular vesicles (EV) are recognized as important mediators of cell-cell communication, capable of transferring various cargoes, including proteins, nucleic acids, metabolites, and even entire organelles, between cells. Thus, EV are potential biomarkers of health status. We hypothesized that heat stress during late gestation would alter the secretion profile of EV at parturition in dairy cows. To test this, we collected maternal plasma samples within 2 h after parturition (n = 10/trt): late gestation heat stress (HT) and active cooling system (CL). The EV were isolated from plasma via single-step size exclusion chromatography. Preparations were checked for quality by transmission electronic microscopy and then quantified by NTA, before proteomic analysis. High-resolution MS and bioinformatics analysis were conducted with ANOVA comparing CL versus HT at a significance threshold of $P \leq 0.05$. The EV biomarkers from bovine plasma have been detected, including several proteins significantly affected when comparing the secretion CL versus HT. We noted increased secretion in the CL group of laminin subunit gamma 1 ($P = 0.001$), $\alpha 2$ ($P = 0.006$), and $\beta 1$ ($P = 0.05$); transferrin receptor protein 1 ($P = 0.02$); collagen IV ($P = 0.03$); fibrinogen α chain ($P = 0.04$), gamma-B chain ($P = 0.05$), and β chain ($P = 0.05$); von Willebrand factor ($P = 0.05$); and SPN protein ($P = 0.05$). Furthermore, in the CL group, the proteins that decreased include immunoglobulin domain lambda ($P = 0.01$) and heavy chains ($P = 0.02$); HGF activator ($P = 0.01$); protein HP-20 homolog ($P = 0.02$); TFG protein ($P = 0.04$); amine oxidase 3 ($P = 0.04$); collectin member 10 ($P = 0.04$). These findings suggest that these EV may play a role in modulating maternal metabolism and immune adaptations at the time of parturition within maternal circulation. In conclusion, late gestation heat stress alters the secretion of specific proteins that are involved in

adaptations related to parturition. Further investigation is needed to better understand the functionality of EV during late gestation and the impact of heat stress on the molecules they carry.

Key Words: extracellular communication, dry period, proteomic

2670 Assessing the associations between behaviors and vaginal temperature of lactating cows in automatic milking system during summer. T. N. Marins*, C. G. Savegnago, J. Gao, Y. B. Amancio, and S. Tao, *Department of Animal and Dairy Science, University of Georgia, Athens, GA.*

Understanding the link between behavior and body temperature is critical to enhance welfare and performance during heat stress. Our aim was to identify associations between behavior and vaginal temperature (VT) of dairy cows during summer. Mid to late lactating multiparous Holstein cows ($n = 45$, parity = 2.3 ± 0.6 ; DIM = 211 ± 31 ; milk yield [MY]: 37 ± 11 kg/d) housed in a freestall barn with guided flow automated milking systems (visit at AMS = 2.3 ± 0.6 /d) were enrolled. Only fans were provided over freestalls and feedline for cooling. The VT was measured every 5 min, and behaviors were recorded every 15 min using wearable sensors (Nedap) for 7 consecutive days. Behavior data were summed by day and daily average (Avg), maximum (Max), and CV of VT within a day were calculated for simple linear regression using PROC GLM of SAS 9.4. Dependent variables included behavioral measures and MY, and independent variables included VT measures. During the study, AvgTHI = 77.4 and MaxTHI = 82.7. Daily lying time (min/d) was negatively associated with the Max ($= 1988 - 32\text{MaxVT}$, $R^2 = 0.02$, $P = 0.03$) and CV of VT ($= 888 - 250\text{CVVT}$, $R^2 = 0.13$, $P < 0.01$), but daily standing time (min/d) was positively associated with the Max ($= -607 + 33\text{MaxVT}$, $R^2 = 0.02$, $P = 0.02$) and CV of VT ($= 530 + 245\text{CVVT}$, $R^2 = 0.13$, $P < 0.01$). Daily MY was positively associated with CV of VT ($= 59 + 27\text{CVVT}$, $R^2 = 0.08$, $P < 0.01$). These may suggest that longer standing time leads to greater heat dissipation and thereby greater VT variation and MY. Daily walking time (min/d) tended to be positively associated with the CV of VT ($= 23 + 4\text{CVVT}$, $R^2 = 0.01$, $P = 0.08$). Daily ruminating time (min/d) was positively associated with the Avg ($= -2,327 + 70\text{AvgVT}$, $R^2 = 0.12$, $P < 0.01$) and Max of VT ($= -2,055 + 62\text{MaxVT}$, $R^2 = 0.12$, $P < 0.01$), whereas daily eating time was negatively associated with the Avg ($= 1,455 - 34\text{AvgVT}$, $R^2 = 0.08$, $P < 0.01$) and Max of VT ($= 1,387 - 32\text{MaxVT}$, $R^2 = 0.09$, $P < 0.01$). In conclusion, variations in VT are associated with lying/standing behavior and MY, whereas hotter cows had greater rumination time.

Key Words: vaginal temperature, behavior, summer

2671 Relationships between physiological responses to heat stress during early lactation. K. Joyce*, T. Baber, S. Newman, S. Yang, and M. L. Rhoads, *Virginia Tech, Blacksburg, VA.*

Individual animal responses to a heat stress insult are highly variable, making it difficult to predict the resultant impact on productivity. Whereas ambient conditions and rectal temperatures are easily measured on-farm, they are not always good indicators of how milk production will be affected. The dairy industry needs more reliable, yet easily measured variables that predict productivity during summer heat stress. This project aimed to examine the relationship between seasonal heat stress, rectal temperatures, blood glucose concentrations, and milk production of lactating Holstein cows ($n = 129$). From May to September, glucose was measured in blood samples collected from the coccygeal vein twice weekly using a handheld glucometer (Abbott Precision Xtra). Rectal temperatures were also collected on sample

days. Milk production information was gathered from farm records (PCDART software; DRMS, Raleigh, NC), and ambient temperature, humidity, and dew point were recorded (EasyLog; Lascar Electronics, Erie, PA). The days surrounding sample collections were categorized as having no HS (0 h above 68 Thermal Humidity Index [THI]), moderate HS ($>0-8$ h above 68 THI), or severe HS (>8 h above 68 THI). Blood glucose concentrations did not differ between THI categories on the day before or the day of sampling. Milk production and rectal temperatures differed based upon the THI category the day before sampling for multiparous cows ($P < 0.05$), but neither variable differed for primiparous cows. Rectal temperatures of all cows differed based upon the THI category on the day of collection ($P < 0.05$). A subset of cows ($n = 28$) was categorized as hypoglycemic or nonhypoglycemic based upon their blood glucose concentrations on no-HS days and severe-HS days (hypoglycemic if sustained reduction on severe-HS days). There was a tendency for primiparous hypoglycemic cows to produce more milk than their nonhypoglycemic counterparts ($P < 0.15$), but milk production did not differ in multiparous cows. Taken together, these results suggest that the predictive capability of these easily measured variables differs between primiparous and multiparous cows.

Key Words: cattle, glucose, heat stress

2672 Effects of prenatal heat stress on dairy heifer calf birth weight in a humid continental climate. M. J. Brower*, L. M. Conrad¹, V. Ouellet², and T. J. DeVries¹, ¹*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada*, ²*Department of Animal Sciences, Université Laval, Laval, QC, Canada.*

Adverse maternal environments can influence prenatal development, affecting calf growth and birth weight. In humid continental climates, dairy cattle experience periods of high temperatures and humidity for several months per year. The impact of late gestational heat stress on calf birth weight in this type of climate has not been well established. Our objective was to determine whether calves exposed to prenatal heat stress during the close-up period have lower birth weight. Late-gestation Holstein dairy cows (42 primiparous, 24 multiparous) were enrolled at a commercial dairy farm in Ontario, Canada during the close-up period (~ 21 d relative to expected calving date) between May and August 2024. Gestation length averaged 276 ± 6 d (mean $\pm$ SD). The farm was visited 3 $\times$ /wk for the study period. Calves were weighed after birth, with calves averaging 1.0 ± 0.9 d of age (range = 0–3) at weighing. Data loggers, placed within pen just above cow level, recorded ambient temperature and relative humidity at 10-min intervals. Temperature-humidity indices (THI) were calculated for each cow's close-up period, including average daily THI, maximum THI, and average h/d for which THI exceeded the thresholds of 62, 65, 68, and 72. Data were analyzed using mixed-effect linear regression models, with calving month as a random effect, and controlling for the effect of dam parity, gestation length, and calf age at weighing. Daily THI averaged 67.4 ± 2.2 (range = 62.1–69.4). The maximum THI averaged 73.0 ± 2.0 (range = 68.0–74.8). The average time per day (and range) above the THI thresholds of 62, 65, 68, and 72, were 19.4 ± 3.1 h/d (12.5–23.0 h/d), 16.0 ± 3.9 h/d (7.2–20.1 h/d), 12.2 ± 3.8 h/d (3.5–16.0 h/d), and 5.7 ± 2.1 h/d (1.1–7.7 h/d), respectively. Calf birth weight averaged 42.1 ± 4.9 kg (range = 32.2–53.5 kg). For each 1-unit increase in daily average THI and maximum THI, calf birth weight ($\pm$ SE) was reduced by 0.77 ± 0.27 kg ($P = 0.007$) and 0.90 ± 0.30 kg ($P = 0.002$), respectively. Each 1 h increase in time (h/d) THI exceeded 62, 65, 68, and 72, birth weight decreased by 0.4 ± 0.2 kg ($P = 0.03$), 0.4 ± 0.2 kg ($P = 0.02$), 0.5 ± 0.2 kg ($P = 0.008$), and 0.9 ± 0.3 kg ($P = 0.001$), respectively. Overall, intermittent exposure to prenatal heat stress reduced calf birth weight, reinforcing the need to

mitigate heat stress during the close-up period in areas with a humid continental climate.

Key Words: heat stress, birth weight, close-up

2673 Late gestation heat stress and OmniGen-AF may alter immune cell populations in dairy cows. J. M. Lance^{*1}, L. T. Casarotto¹, H. Olmo¹, S. Casaro¹, K. M. Glosson², B. D. Humphrey², J. D. Chapman², J. P. Driver³, C. D. Nelson¹, and G. E. Dahl¹, ¹University of Florida, Gainesville, FL, ²Phibro Animal Health Corporation, Teaneck, NJ, ³University of Missouri, Columbia, MO.

Heat stress (HT) of dry dairy cows impairs immune function, and cortisol (COR) may be a factor. Parturition also alters peripheral blood leukocytes with inconclusive patterns reported. Previous work indicates OmniGen-AF (OMN) mitigates the effects of HT on immune function. To test this, dry cows were fed OMN and electric blankets (EB) were used to induce HT. We hypothesized OMN would ameliorate the effects of COR on the immune system in response to EB-induced HT. At dry-off, 50 Holstein cows were fitted with EB or not to simulate cooled conditions (NB). Cows were fed OMN (56 g/d) or no OMN (CON), resulting in a 2 × 2 factorial of 4 treatments (TRT): EBCON (n = 13), NBCON (n = 13), EBOMN (n = 12), and NBOMN (n = 12). Serum COR and heat-shock protein (HSP) were measured in a subset of cows (n = 9–10/TRT) at enrollment (d 50), 14 d, and 7 d (±3 d) before calving. Lymphoid (n = 6/TRT) and myeloid (n = 3–6/TRT) cell populations were assessed by flow cytometry on a subset of cows at d 50, d 14 (±3 d) pre-, and d 2 and 7 postpartum (±3 d). The EB-induced HT was confirmed by elevated rectal temperature and respiration rate compared with NB groups. The COR concentrations tended to be higher on d 7 (P = 0.09) versus d 50 and d 14. The HSP was higher (P = 0.03) in EB cows compared with NB cows. Expression of activation marker CD62L on B-cells and cytotoxic T-cells decreased leading up to parturition (P < 0.01), but only B-cells remained more activated after calving (P = 0.02). The PMN cells were activated (less CD62L, more CD11b) after d 50 (P < 0.05) and expression of CD11B remained higher after calving (P = 0.03). B-cell CD62L followed the same pattern (P < 0.05). Expression of activation marker CD11B on monocytes decreased (P = 0.04) and tended to decrease on B-cells (P = 0.07) in EBCON. Cell activation was rescued in EBOMN and was comparable to NBCON and NBOMN. Cows fed OMN had higher (P = 0.02) proportions of αβ T-cells and decreased γδ T-cell MFI (P = 0.01) compared with CON. The γδ T-cell MFI and those expressing CD62L were decreased (P < 0.01) leading up to parturition and did not recover by d 7 (P < 0.05). The COR was low and did not drive immune cell changes, but OMN and HT impacted immune cell populations.

Key Words: electric blanket, flow cytometry

2674 Factors related to long-term stress in dairy cattle and the impact of that stress on milk yield and reproductive outcomes. J. F. Gille¹, S. G. Schoenfeld¹, A. Schaik¹, M. R. Lauber¹, P. M. Fricke¹, A. Bjurström¹, M. P. Lippert¹, T. L. Kohlman¹, J. McCarville¹, A. Ulness¹, J. Guyader², M. C. Wiltbank¹, L. L. Hernandez^{*1}, and P. L. J. Monteiro^{3,1}, ¹University of Wisconsin–Madison, Madison, WI, ²Evonik Operations GmbH, Essen, Germany, ³University of Florida, Gainesville, FL.

We aimed to assess factors associated with long-term stress in dairy cattle and impact on milk production and reproductive outcomes. From July to September 2023 (summer) and January to March 2024 (winter), 32 dairy farms in Wisconsin were visited. On d 32 ± 3 after the first service (113 ± 0.53 DIM), hair samples were collected from the tail

switch of lactating primiparous (n = 284) and multiparous (n = 559) cows. A total of 30 cows per farm (15 per season) were sampled. Body condition score (1–5), hygiene score (HySc, 1–3), hock score (HoSc, 1–3), and locomotion score (LoSc, 1–3) also were assessed. Hair cortisol was extracted and analyzed using ELISA. Data were evaluated using the GLIMMIX procedure in SAS. Hair cortisol levels varied significantly among farms (ranging from 7.0–38.7 pg/mg). Parity had no effect on cortisol levels (P = 0.27). Cows had increased cortisol concentrations in summer compared with winter (19 ± 2.0 vs. 16 ± 1.7 pg/mg; P = 0.02). Cows with BCS ≤ 2.75 had significantly higher cortisol levels than those with BCS 3.00 or ≥ 3.25 (20 ± 2.3 pg/mg vs. 15 ± 1.7 pg/mg vs. 17.3 ± 1.9 pg/mg; P < 0.01). Cows with HoSc 1 had reduced cortisol levels compared with those with HoSc ≥ 2 (14 ± 0.5 pg/mg vs. 20 ± 3.3 pg/mg; P = 0.05). No significant associations were detected between cortisol levels and LoSc (P = 0.74) or HySc (P = 0.24). Cows were categorized into high- and low-cortisol groups within each farm based on the median cortisol concentration. Multiparous cows with high cortisol levels tended to produce less milk than those with low cortisol (14,138 ± 330.3 pg/mg vs. 15,062 ± 326.2 kg/lactation; P = 0.10), whereas no difference was observed in primiparous cows (P = 0.81). Hair cortisol was not associated with pregnancy per AI on d 30 (P = 0.36) or d 60 (P = 0.86), or with pregnancy loss (P = 0.86). Thus, long-term stress levels varied among farms. Multiparous cows with increased cortisol tended to produce 900 kg less milk, and no association was observed related to reproductive outcomes. Acknowledgments: Evonik Operations GmbH.

Key Words: chronic cortisol, milk production, score

2675 Precalving nutritional factors affecting colostrum quality and yield in Eastern Canadian dairy herds. K. Dubois^{1,2}, C. Côrtes², S. Buczinski¹, and M. Duplessis^{*3}, ¹Département des Sciences Cliniques, Université de Montréal, Saint-Hyacinthe, QC, Canada, ²Agrinova, Alma, QC, Canada, ³Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada.

The study aimed to identify precalving dietary ingredients, nutrient composition, cow characteristics, and herd management affecting colostrum immunoglobulin G (IgG) concentration and yield in Canadian Holstein dairy farms. A total of 364 multiparous cows in 51 herds were blood sampled between 0 to 33 d precalving. Precalving dietary ingredients were sampled and their quantities offered were recorded. Colostrum samples at the first milking after calving were collected. To reduce the number of interdependent variables to include in the multivariable models, a principal component analysis was carried out on data relative to the ration. Univariable models were performed with colostrum yield and colostrum IgG and Brix values as dependent variables. Independent variables leading to P < 0.20 at the univariable level were retained in the multivariable linear regression model, followed a backward elimination by using PROC MIXED of SAS with herd as a random effect. Colostrum yield, IgG, and Brix averaged 5.9 (SD = 3.9) kg, 56.1 (23.7) ng/mL, and 0.24 (0.05), respectively. Colostrum IgG and Brix led to the same final models. Plasma nonesterified fatty acid concentration, time between calving, and the first milking were positively related to colostrum yield. A dry period length of 65 d and more (6.7 [95% CI: 5.9–7.5] kg) tended to lead to a greater colostrum yield than a dry period length of 51 d and less (5.7 [95% CI: 5.0–6.5] kg; P = 0.08). Decreasing prepartum dietary fiber, ash, Ca, Mg, and Fe and increasing energy were related to greater colostrum yield (P < 0.05). Second parity cows had lower colostrum IgG concentrations than third and more parity cows. Colostrum IgG was negatively related to mineral and protein supplements and positively with commercial energy supplements (P < 0.01). A negative relationship was observed between colostrum IgG and time between

calving and colostrum collection ($P < 0.01$). The model including diet, cow, and herd management factors explained, respectively, 47%, 34%, and 40% of the colostrum yield, IgG, and Brix variability. In summary, the studied factors explained a significant part of the colostrum yield and IgG variability.

Key Words: cattle, immunoglobulin, calf

2676 Maternal late gestation heat stress modifies skin characteristics of great-granddaughters. B. D. Davidson*, K. E. Hardy, and J. Laporta, *University of Wisconsin–Madison, Madison, WI.*

Homeostasis and thermoregulation depend on the interplay of the hair coat and skin. Maternal late gestation heat stress triggers postnatal hair and skin adaptations in her daughters and granddaughters. Herein, we investigated the potential transgenerational effects of late gestation heat stress on the hair and skin morphology of the great-granddaughters. Pregnant dams (F_0) were heat stressed (access to shade) or cooled (access to shade, fans, and water soakers) during the last ~56 d of gestation in summer. The first generation of heifers (F_1) that experienced in utero heat stress or cooling (HT_{F_1} , $n = 36$; CL_{F_1} , $n = 37$) were managed as a single cohort until they gave birth to the second generation (F_2) that experienced heat stress or cooling (HT_{F_2} , $n = 12$; CL_{F_2} , $n = 18$) as a germ cell within the F_1 fetal ovaries. All F_2 heifers were managed identically as a cohort, until they gave birth to the third generation (F_3 , great-granddaughters) that were “unexposed” to the initial F_0 treatments (HT_{F_3} , $n = 9$; CL_{F_3} , $n = 11$). At 70 d of age, a hair sample and skin biopsy were collected from the neck on a subset of F_3 heifers ($n = 6$ /group). Hair length and diameter, skin epidermis thickness and area, dermis thickness, sweat gland (SWG) number, area, and depth, and sebaceous gland (SBG) number, area, and depth were evaluated using H&E staining and analyzed in ImageJ. Data were analyzed using PROC MIXED in SAS with main effects of F_0 treatments. No differences were captured in hair length or diameter, epidermis thickness or area, depth of the shallowest SWG, the depth of the SBG, dermis thickness, and the number or size of SWG. However, relative to CL_{F_3} , HT_{F_3} had fewer (13.3 vs. 17.8 ± 1.1 glands, $P = 0.02$; HT_{F_2} vs. CL_{F_2} , respectively) and smaller SBG ($61,641$ vs. $89,963 \pm 6,768 \mu m^2$, $P = 0.01$). Although the SWG number or size did not differ, the distance from the skin surface to the deepest SWG tended to be shorter in the HT_{F_3} (1.1 vs. 1.3 ± 0.04 mm, $P = 0.07$). Overall, late gestation heat stress alters sebaceous gland size and number, and sweat gland location within the dermis of F_3 heifers, suggesting a transgenerational programming of postnatal thermal adaptivity.

Key Words: hyperthermia, multigenerational

2677 Exploratory analysis characterizing the association between late-lactation milk yield or somatic cell count and rectal temperature after dry-off in dairy cows. E. Leonard*, C. Florentino, M. Ruch, A. Pereira, and L. Caixeta, *Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, MN.*

Rectal temperature (RT) indicates systemic inflammation that is often linked to pain and welfare concerns. After dry-off (DO), inflammatory pathways drive udder involution, mammary gland remodeling, and immune response to infections. Milk yield and SCC may influence these processes, potentially affecting RT dynamics. Thus, the objective of this exploratory study is to determine whether milk yield or SCC are associated with changes in RT post-DO. The RT was measured in 140 dairy cows 1 d post-DO and before vaccination using a digital thermometer. Cows with $RT > 39.1^\circ C$ were classified as having a fever.

All cows were housed in the same pen at 1 Minnesota dairy farm, and data were collected during the months of January and February of 2025. The average daily milk yield during the week before DO (MLKDO) and SCC from the most recent DHIA test were obtained from farm management records. Cows were dichotomized into low SCC ($< 200,000$ cells/mL) or high SCC ($> 200,000$ cells/mL) for analysis. The association of MLKDO and SCC with RT were analyzed using separate multiple linear regression and Spearman correlation models. All models accounted for the fixed effects of lactation, days carrying calf, days in milk, and either SCC or MLKDO, as appropriate. Interestingly, 19.3% of cows exhibited a fever the day post-DO. Among all primiparous cows ($n = 53$), 9.4% exhibited fever, whereas 27.3% and 24.1% of all secondiparous ($n = 33$) or multiparous ($n = 54$; 3+ lactation) exhibited fever, respectively. Neither MLKDO ($P = 0.36$) nor SCC ($P = 0.63$) were associated with RT post-DO. Spearman correlation analysis revealed a weak negative correlation between MLKDO and RT ($r = -0.08$) and between SCC and RT ($r = -0.12$). In this exploratory analysis, fever in dry cows was not explained by MLKDO or SCC. Further research is necessary to understand factors contributing to temperature fluctuations at DO. Providing insights into physiological adaptations during this transition can inform management strategies to promote cow health and productivity.

Key Words: fever, inflammation, dry period

2678 Understanding “exciting opportunities” and “biggest concerns” in the Vermont dairy community. B. M. Craig^{*1,2}, D. S. Conner², and H. M. Dann¹, ¹*William H. Miner Agricultural Research Institute, Chazy, NY*, ²*University of Vermont, Burlington, VT.*

Understanding the point of view of dairy industry stakeholders can give context to how the industry can prepare for environmental, social, and economic challenges. To address this, an online survey was designed using Qualtrics. Disseminated at both the Vermont Dairy Producers Conference and the Vermont Organic Dairy Days, stakeholders were asked to list “exciting opportunities” and “biggest concerns” about the industry. Eighty responses were collected from stakeholders, 24 of whom identified as dairy producers. Using thematic analysis, main themes were identified corresponding to “exciting opportunities” and “biggest concerns.” The themes derived from “exciting opportunities” included technical advancements, diversification, and evolving consumer opinions. Responses within the technical advancement theme included discussions of precision agriculture, use of artificial intelligence, and technical improvements for animal health and welfare. Diversification yielded responses including beef on dairy, specialty dairy products, and carbon markets. Responses within the theme of evolving consumer opinion indicated an increased awareness of the health benefits from dairy foods, and an increased desire from the public to understand dairy farming. The themes derived from the “biggest concerns” included viability and the producer-consumer relationship. Responses within the theme of viability included questioning the economic sustainability of dairy farming, land access challenges, and succession. Responses within the theme of producer-consumer relationships included loss of consumer trust, sustainability demands, and competition from nondairy products. These themes can provide insight to dairy extension teams, marketing groups, and policymakers of the nuanced balance between opportunities and constraints for actors within the industry. From both sets of themes, we can see that interactions between consumers and the dairy industry are a source of concern and possibility. Future research could investigate the synergies within these relationships.

Key Words: thematic analysis, qualitative research, industry trend

2679 Comparing manual and computer vision-based body condition scores in Holstein and Jersey breeds according to the lactation stage. T. Abdulrahman^{*1}, B. B. Medeiros¹, C. Saha¹, J. Hooker¹, and A. A. C. Alves^{1,2}, ¹*Department of Animal and Dairy Science, University of Georgia, Athens, GA*, ²*Institute for Integrated Precision Agriculture, Athens, GA*.

Body condition score estimates body fat reserves and energy balance in dairy cows. Periodic BCS monitoring is crucial, as it reflects energy balance changes affecting milk production, reproductive performance, and overall health. This study investigated the relationship between manual BCS and automated BCS predicted using computer vision (CVS_BCS) in relation to milk yield and across lactation periods (early, mid, and late lactation) in Holstein (H) and Jersey (J). A total of 8,155 images (4,340 for H and 3,815 for J) were collected using an Intel RealSense D435 camera. Animal IDs were retrieved using RFID information. Collected depth images were segmented using a U-Net architecture. Two trained observers scored BCS on a 1-to-5 scale over 5 mo. A ResNet50-backbone model with focal loss was used to handle class imbalance and hard training examples. We used a restricted 5-fold cross-validation to assess model accuracy, ensuring that all data from the same cow were assigned exclusively to either the training or validation folds to prevent data leakage. Training on both breeds and validating separately using the depth images provided the best accuracy for prediction, with an accuracy of (92.4%) at a 0.5-unit deviation for Holstein and 88.8% for Jersey. Linear models were used to analyze the relationship between observed BCS and predicted BCS with respect to DIM, lactation number, and breed. Both variables (BCS and CVS-BCS) showed a significant linear relationship with DIM ($P < 0.001$), but lactation number and breed did not significantly influence automated or manual BCS ($P > 0.05$). The models explained 16.3% and 20.8% of BCS and CVS-BCS, respectively. The correlation between predicted BCS and milk yield was negative (-0.43), similarly to that between manual BCS and milk yield (-0.39), indicating that as milk production increases, BCS decreases due to energy utilization. Our results suggest that CVS-BCS could replace manual BCS for data-driven management decisions, as they showed the same association pattern across lactation stages and with milk yield.

Key Words: deep learning, depth image, smart management

2680 Evaluation of an ear-tag accelerometer sensor for preweaned dairy calves. B. J. Heins^{*}, I. W. Haagen, and K. T. Sharpe, *University of Minnesota, St. Paul, MN*.

The objective of this study was to evaluate an ear-tag accelerometer sensor and correlate behavior with an automatic calf milk feeder system for preweaned dairy calves. The study was conducted at the University of Minnesota West Central Research and Outreach Center, Morris, MN, dairy. Calves were Holstein ($n = 4$), GrazeCross crossbreds ($n = 7$) composed of the Normande, Jersey, and Viking Red breeds and ProCross crossbreds ($n = 16$) composed of the Montbéliarde, Viking Red, and Holstein breeds and were born from September 2024 to November 2024. During the preweaned phase, activity, rumination, and eating behavior were monitored electronically using an ear-tag accelerometer sensor (CowManager SensOor, Agis Automatisering BV, Harmelen, the Netherlands). Calves were introduced on d 5 to the Holm & Laue HL100 Programmable Calf Feeder (Holm & Laue GmbH & Co. KG, Westerronfeld, Germany). The automated calf feeder had a radio frequency identification reader placed on the left side of the hygiene stall and scale for calf identification. All calves were fed 10 L/d of milk. Calves were weaned from the automated feeder at 84 d. Data were downloaded from the CalfGuide management software. The

sensor detected and identified ear and head movements, and through algorithms, the sensor classified each minute as one of the following behaviors: rumination, eating, not active, active, and high active. The associations of the drinking speed from the automated feeder and sensor measurements were determined with Pearson correlation (PROC CORR of SAS). Furthermore, PROC GLM of SAS was used to regress sensor measurements on drinking speed and daily milk consumption for each day. A total of 1,605 observations were used for statistical analysis. The Pearson correlation of ruminating and drinking speed (mL/min) was 0.499 ($P < 0.001$), and eating and drinking speed was 0.409 ($P < 0.001$). The correlation of not active and drinking speed was -0.51 ($P < 0.001$). Correlations of ruminating and drinking speed for individual calves ranged from 0.09 to 0.744 ($P < 0.001$). The slope of the regression line was 0.21 ($T < 0.01$) for ruminating, 0.109 for eating, -0.04 for active, and -0.36 for not active behaviors. The results suggest that there are ear-tag sensors to detect behaviors in preweaned calves to support management decision making.

Key Words: automated feeder, calf, precision technology

2681 Predictive models for the classification of bovine ingestive behavior in heterogeneous databases. M. A. C. Danes^{*}, L. Henrique Silva, V. A. Oliveira, D. S. Souza, M. C. S. Santos, and F. D. de Jesus, *University of Lavras, Lavras, MG, Brazil*.

The random forest predictive model is widely used in precision livestock farming, but more complex models may perform better with more complex databases. Thus, we aimed to compare the performance of 4 predictive models in classifying bovine ingestive behavior. For this, 5 DB were collected. The first was obtained from a farm using a tiestall system. The second was collected from a farm using a freestall system. The third originated from a pasture-based property, all involving dairy cows. The fourth DB was collected from beef cattle housed individually. Finally, the fifth DB was obtained from a farm with dairy cows in a compost barn system. For model training, the first 4 DB were unified, whereas the fifth DB was used for external validation. The cattle were equipped with triaxial accelerometer and gyroscope sensors attached to the nape, recording data every second. Visual observations were conducted to label behaviors as “feeding” or “not feeding.” The predictive models evaluated were random forest (RF), gradient boosting (GB), artificial neural network (ANN) of the multi-layer perceptron (MLP) type, and ANN of the single layer perceptron (SLP) type. Performance metrics included accuracy, precision, and F1-score. The best-performing model was MLP (Table 1). This model, being more complex, demonstrated superior predictive capacity for handling heterogeneous data. In intermediate performance, the RF and GB models stood out. The SLP model, on the other hand, obtained the lowest results, indicating difficulty in predicting ingestive behavior. The difference between MLP and SLP, both ANN models, reinforces the need to select predictive models suited to the available data. The results highlight that more complex predictive models performed better in classifying bovine ingestive behavior across

Table 1 (Abstr. 2681). Performance metrics for the different predictive models adopted

Predictive model	Accuracy (%)	Precision (%)	F1-Score (%)
Random forest	75.1	65.1	35.5
Gradient boosting	74.1	61.1	31.0
Multi-layer perceptron	77.9	79.5	42.2
Single layer perceptron	62.6	36.4	40.2

different production systems and remained effective when validated with external data.

Key Words: animal behavior, machine learning, precision livestock farming

2682 Evaluation of a nonantibiotic, no-withdrawal, nonprescription therapy at dry-off to lower somatic cell count. B. J. Heins^{*1}, K. T. Sharpe¹, P. Pinedo², V. E. Cabrera³, E. M. Silva³, R. A. Lynch⁴, Q. K. Kolar⁴, A. De Vries⁵, E. K. Miller-Cushon⁵, and G. M. Schuenemann⁶, ¹University of Minnesota, St. Paul, MN, ²Colorado State University, Fort Collins, CO, ³University of Wisconsin, Madison, WI, ⁴Cornell University, Ithaca, NY, ⁵University of Florida, Gainesville, FL, ⁶Ohio State University, Columbus, OH.

The use of antibiotics, including dry cow therapy, is prohibited for organic livestock production in the United States. Therefore, alternative management practices that maintain udder health need to be identified. The objective of this study was to compare the use of ozonized and stabilized food grade extra pure corn germ oil (Ozolea-Mast; Castegnato BS, Italy) for a method of dry cow therapy in Holstein and crossbred cows. Cows were housed at the University of Minnesota West Central Research and Outreach Center, Morris, MN, and data were collected from August 2023 to April 2024. Holstein cows (n = 21), GrazeCross crossbred cows (n = 6) composed of the Normande, Jersey, and Viking Red breeds, and ProCross crossbred cows (n = 40) composed of the Montbéliarde, Viking Red, and HO breeds were included in the study utilizing 528 quarter samples. The 67 cows were randomly assigned to 2 treatments: Ozolea (n = 33) or antibiotic (n = 34) therapy at dry-off. Cows were balanced by lactation number, breed, and expected calving date. Cows had no visible signs of clinical mastitis at the time of dry-off. The Ozolea cows were provided with 1 tube per quarter for the second to last milking and 1 tube per quarter at the last milking before dry-off. The antibiotic therapy was cephalixin benzathine broad spectrum antibiotic provided as 1 tube per quarter at last milking before dry-off. Aseptic quarter milk samples were collected at dry-off and at calving for bacteriological culture. Cows were followed for 5 mo after calving and SCC were recorded from DHIA test day. Independent variables for statistical analysis were breed group, parity, and month after calving. After calving, there were no significant differences ($P > 0.05$) in no growth from milk cultures for the 2 treatment groups for each quarter. Somatic cell count was similar ($P > 0.05$) for both treatment groups across the first 5 mo of lactation (197 for Ozolea vs. 123 for antibiotic therapy). Milk production was no different ($P > 0.05$) 26.8 kg/d for Ozolea cows and 26.1 kg/d for antibiotic therapy cows. In conclusion, there were no differences between treatment groups on curing the probability of intermammary infection during the dry period.

Key Words: organic, mastitis, dry-off

2683 Investigating dairy cattle welfare on Iranian intensive farms using resource-based measures: Cow flow management. A. Jafari-Gh^{*1}, R. Laven¹, F. Khaloubagheri², S. M. H. Mirrahimi³, S. Jafari-Gh⁴, K. R. Mueller¹, M. Dehghan Banadaky⁵, and E. Vallee¹, ¹School of Veterinary Science, Massey University, Palmerston North, New Zealand, ²Independent Researcher, Palmerston North, New Zealand, ³School of Veterinary Medicine, Karaj Islamic Azad University, Karaj, Iran, ⁴Animal Science Department, College of Agriculture and Natural Resources, Karaj Islamic Azad University, Karaj, Iran, ⁵Animal Science Department, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.

The change from pastoral systems to housed systems has resulted in substantial challenges for the dairy cattle industry including issues regarding cow flow and handling. In Iran, most of the milk consumed is produced on intensive dairy farms. Yet, there is no official welfare assessment scheme or routine welfare assessment on Iranian dairy farms. Thus, we designed and used a welfare-assessment protocol that included resource-based welfare measures, such as measures related to cow flow during milking time. The protocol was used in 28 freestall (FS) and 34 bedded-pack (BP) farms in Iran in 2022 and 2023. Measurements were done when no cows were in the tracks. Descriptive statistics were calculated using SAS version 9.4. Results show that out of 48 farms, the transfer passage (from pen to parlor) condition was good (i.e., no holes, bumps, or slippery surface) in 16 FS and 9 BP farms, moderate (holes with <10 cm diameter but no slippery surface) in 6 FS and 2 BP, and bad (large holes and slippery surface or hazards) in 15 farms (7 FS and 8 BP). Additionally, 8 small BP farms (small: <180 lactating cows) kept the cows in pens that were next to the parlor, so there were no transfer lanes to use. Cows were milked 3 times a day in 94% of farms and spent a maximum of >3 h/d for milking in 52% of farms, with 26% of them spending ≥ 4 h/day for milking (n = 54). Additionally, cows stood on concrete floor in 74% of holding areas. Lack of use of rubber mats can negatively impact the welfare of the cows, as cows can increase the incidence of claw horn hemorrhage and lameness. Track widths (median of 118 cm for small, 185 cm for medium, and 265 cm for large farms) did not meet the standards. In addition, even though the median entrance/exit gradient was 5%, parlor entrance was steep (i.e., 10%–15%) in 12.5% and too steep (i.e., >15%) in 3.8% of farms, whereas exit was steep in 18% and too steep in 3.4% of farms. Results show a lack of attention to structural issues that can directly influence cows' welfare. This lack of attention can lead to poor cow handling and increased lameness on these farms.

Key Words: cattle welfare, farm management

2684 Impacts of volatile organic compounds from wildfire smoke on dairy cow metabolism and production. A. Pace^{*1}, N. A. C. Johnston², A. Anderson^{1,3}, C. Sia², D. D. Miller², M. Larson¹, K. Mirkin^{1,3}, P. Villamediana¹, and A. L. Skibieli¹, ¹University of Idaho, Moscow, ID, ²Lewis-Clark State College, Lewiston, ID, ³Washington State University, Pullman, WA.

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

Wildfire smoke contains particles (PM) and volatile organic compounds (VOC). Wildfire-PM reduces milk production and alters metabolism in cattle, but the contribution of wildfire-VOC is unknown. Lactating (2019–2022; average DIM = 21 d; n = 43) and dry (2019–2021; n = 41) Holstein cows were followed across wildfire seasons. Temperature-humidity index (THI) and PM were recorded. Passive thermal desorption tubes were used to collect VOC, which were analyzed using GC-MS. Weekly BW, BCS, rectal temperature (RT), and respiratory rate (RR) were recorded, and blood was collected to quantify plasma glucose (GLU), BHB, and nonesterified fatty acids (NEFA). Lactating cow milk yields were recorded, and composition was analyzed. Gestation length, calf birth weight (CBW), and CBW relative to gestational age (CBWA) were recorded for calves born to dry cows. Principle component (PC) analysis was performed on 24 VOC, PM, and THI. The top 4 PC, parity, and year were fixed effects in mixed models, with repeated effect of time within cow, and lags of up to 21 d. Calf data were analyzed using mixed models with year, sex, and dam parity as fixed effects, and a random effect of cow. Wildfire smoke was present in all years except 2019, which had low VOC and PM concentrations. Top contributors to

PC were PM, toluene, and heptane (PC1), trichloromonofluoromethane (PC2), benzene species (PC3), and THI (PC4). During exposure to PC2 and 4, lactating cow RR increased ($P < 0.03$). Lactating cow RT was increased, although BW was decreased during, 14, and 21 d after exposure to PC4 ($P < 0.03$). Lactating cow GLU and NEFA increased during exposure to PC1, whereas BHB increased 7 d after ($P < 0.05$). Milk yield decreased with higher PC1–4 in 2020, PC1 and 4 in 2021, and PC2 and 3 in 2022 ($P < 0.05$). Milk SCC increased with PC2 and 3 only in 2021 ($P < 0.005$). Milk protein percent increased 7 d after exposure to PC1 and 2 ($P < 0.03$). During exposure, PC3 increased dry cow RR ($P < 0.02$). Dry cow BHB increased with PC1–3 ($P < 0.01$). In wildfire years, CBWA was lower than in 2019 ($P < 0.0001$). The VOC and PM appear to be important drivers of altered metabolism and production in cows exposed to wildfire smoke.

Key Words: pollution, air quality, metabolism

2685 Smart Farm Hub: An extension initiative informed by dairy farmer perceptions of precision dairy technologies.

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We developed an extension initiative to provide farmers and consultants with knowledge for adopting and managing precision dairy technologies (PDT), such as sensors and robots. The initiative was based on an ongoing 128-question web survey evaluating US dairy farmers' perceptions and expectations of PDT. Respondents provided herd size, and if PDT were adopted. Furthermore, respondents were asked to use a 5-point Likert scale to indicate their agreement score (AS; 1 = strongly disagree; 5 = strongly agree) to statements designed to assess their perceptions and expectations associated with PDT adoption. Data were summarized in SAS for descriptive purposes. The UNIVARIATE procedure divided the herds into small (SM; ≤ 90 cows; $n = 16$), medium (MD; $90 < \text{cows} < 600$; $n = 28$), and large (LG; ≥ 600 cows; $n = 16$), and the MIXED procedure was used to determine the relationship between herd size and AS. Fifty-eight respondents completed the ongoing survey representing a total of 36,030 cows. Herds ranged from 15 to 9,000 cows, with a mean $\pm$ SD of $621 \pm 1,307$ cows and a rolling herd average of $11,756 \pm 2,293$ kg/yr. Furthermore, 84.5% of respondents indicated the adoption of at least one PDT on their farm. Respondents agreed that PDT adoption improves on-farm decision making (mean $\pm$ SEM; AS = 3.9 ± 0.2), were neutral (AS = 3.0 ± 0.2) about the complexity of data generated by PDT, and expected the adoption of PDT to improve the service provided by consultants (AS = 4.4 ± 0.1). Perceptions and expectations were not affected by farm size ($P > 0.36$). Farm size affected perceptions of educational resources on PDT, with large and small farms agreeing that current resources are limited (AS_{SM} = 3.5 ± 0.3 ; AS_{MD} = 2.5 ± 0.2 ; AS_{LG} = 3.9 ± 0.3 ; $P < 0.01$). Based on the results, a website (smartfarm.cals.wisc.edu) and social media page named Smart Farm Hub were created under the University of Wisconsin–Madison to host informative articles and short videos about PDT adoption and management. Webinars, workshops, and podcasts will provide educational resources on PDT. Future research should evaluate the impact of these educational measures on producer perception and successful PDT adoption.

Key Words: survey, remote sensing, artificial intelligence

2686 Survey-based insights for developing a digital ration formulation tool.

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Intensifying animal productivity through improved nutrition enhances feed efficiency and reduces methane emission intensity. However, smallholder dairy farmers often lack technical support, limiting their adoption of optimal feeding strategies. Digital solutions, such as ration formulation applications, provide farmers with accessible, data-driven recommendations to improve feeding efficiency and profitability. Yet, effective tool development requires a deep understanding of smallholder systems and practices. Thus, we aimed to characterize dairy systems and feeding practices across diverse smallholder contexts. We conducted farm visits in 5 countries: Ethiopia, Thailand, Indonesia, the Philippines, and Vietnam, collecting 235, 41, 38, 35, and 30 survey responses, respectively. Farm visits were planned to reflect regional dairy production contexts, and the survey contained questions regarding property size, herd, milk production, housing system, and feeding system. Farm size ranged from 0.47 ha (Indonesia) to 49.5 ha (Vietnam). Herd size averaged 551 lactating cows in Vietnam, followed by the Philippines (36), Thailand (30), Ethiopia (10), and Indonesia (9). Vietnam had the highest proportion of farms producing over 500 L/d (50%), and the highest use of TMR (63.3%). In contrast, individual ingredient feeding was dominant in Indonesia (100%) and Ethiopia (68.4%), whereas forage + concentrate in the Philippines (76.5%) and Thailand (46.3%). Use of ration formulation software ranged from 0.4% in Ethiopia to 40% in Vietnam and was absent in Indonesia. This suggests that access to feeding technology varies, necessitating adaptable solutions. The survey results provide insights into farm characteristics and level of technology adoption. Regions with larger farms and prior software use may benefit from advanced features, whereas others will require simplified interfaces, input fields, and prior training. Future research will investigate the design and use of the application, ensuring it provides practical, accessible, and region-specific recommendations, ultimately improving feed efficiency, profitability, and sustainability in dairy production.

Key Words: dairy innovation, feeding practice, sustainability

2687 Safety and emergency response training for Hispanic dairy farm employees.

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Dairy farms experience high injury, illness, and employee turnover rates. Farms employing foreign-born Hispanic workers face higher risks due to language barriers and varied educational backgrounds. This project trained 180 Hispanic dairy workers from 9 Midwest farms on workplace safety and emergency preparedness. Researchers initially obtained instructor certifications from the Red Cross, Stop the Bleed, and Fire Safety programs. Interactive Spanish-language workshops combined theoretical sessions (covering key hazards, including animal handling, biosecurity measures, zoonotic diseases, and manure management) with hands-on training (first-aid, fire extinguisher use, CPR, and tourniquets). We aimed to measure the impact of hands-on training on employees' safety knowledge and evaluate their perceptions of the workshop. Participants completed a 10-question pre- and posttraining knowledge assessment. Paired-sample *t*-test analysis indicated significant improvement (pretest: 5.1 ± 2.0 ; posttest: 7.2 ± 1.6 ; $P < 0.001$), demonstrating a large effect size (posttest scores improved by more than 1 SD). Biosecurity and zoonosis topics showed the greatest improve-

ment. Additionally, hands-on first-aid training using mannequins and realistic devices, particularly the “Stop the Bleed” protocol and CPR, significantly increased participants’ knowledge. Animal handling was an area with extensive prior employee experience (79% correct pretest), whereas manure management posed a greater safety concern, with only 25% awareness even after training. All workers successfully learned how to inspect and use fire extinguishers. Employees unanimously rated the training highly useful and demonstrated improved emergency response capabilities. This approach effectively enhanced safety awareness among dairy farm workers regardless of education or experience, potentially helping to increase safety awareness and reduce injury severity. Health and safety educational interventions are thus essential in improving workers’ knowledge, perceptions, and self-efficacy in emergency preparedness. Funded by HICAHS-CSU (HHS-CDC; G-20392–02).

Key Words: dairy safety education, Hispanic worker, first-aid, hands-on

2688 Farmers unwilling to adopt a new culling strategy to reduce Johne’s disease and improve economic sustainability on dairy farms. W. Hull*¹ and J. M. Smith², ¹*University of Vermont, Burlington, VT*, ²*University of Vermont, Burlington, VT*.

Johne’s disease is an infectious disease common within the dairy industry caused by the bacterium *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Johne’s disease has a negative economic impact on dairy herds due to milk production loss, premature culling, and reduced cull values. This study partnered with 8 dairy farms in Vermont in a participatory research project to understand how the provision of information influenced the decision-making process around culling early lactation animals as a means to control Johne’s disease within the herd. Producers were provided with 3 key pieces of information to support the decision-making process, including Johne’s disease status, economic value of the cow, and average milk production, and their subsequent culling choices were observed. Of the 442 individual animals tested, 37 from 4 farms were positive for Johne’s disease. Of farms with these positives, 2 chose to cull animals that were positive and had a negative economic value, whereas none of the farms were willing to cull an animal based solely on low milk production or negative economic value alone. Results from this study show that low production in early lactation is not a strong predictor of Johne’s disease test positivity, and that economic concerns influence but do not solely determine culling decisions. A focus group further explored producer concerns and barriers to adopting Johne’s disease management strategies. Findings suggest that although Johne’s

disease remains a concern, existing management practices and financial constraints shape farmers’ decision-making processes.

Key Words: Johne’s disease, control, extension

2689 Assessment of the level of satisfaction and preference with immersive educational technologies as a training tool for Spanish-speaking dairy farm workers. A. A. Barragan*, R. Sorto, J. Garcia Pruduencio, J. Brown, D. Getz, N. Campos, and L. Periconi, *Department of Veterinary and Biomedical Sciences, Penn State University, State College, PA*.

Immersive educational technologies (IET) have gained attention as innovative educational tools that enhance engagement and interactivity, offering a novel training approach for the dairy industry. This study aimed to evaluate the use of an IET to train dairy farm workers on milking routines. Spanish-speaking milkers (n = 22) from 3 large dairy farms located in central Pennsylvania participated in this study. Participants completed a short pretraining survey (6 questions) to assess their prior experience with VR technology. Afterward, they engaged in a 10-min training session, where a 360° video, of a milker milking a cow, with visual aids clearly describing each step of the milking routine, was displayed through VR goggles. After the training, the employees completed a 5-point Likert scale survey (11 questions) to assess their level of satisfaction and preference with the training program. Data were organized in an Excel file, and descriptive data were presented. When participants were asked if they had previous experience with the technology, 86.4% of workers stated not having any. With regard to the level of comfort with VR sets, 75% of participants agreed or strongly agreed that the VR goggles were comfortable, and 85% reported that they didn’t feel nauseous or sick while using them. Although 86.3% of participants reported that this technology would be beneficial for their learning, only 25% agreed or strongly agreed that they preferred VR over hands-on educational activities. On the other hand, although participants were asked if they would prefer to use this technology in combination with hands-on programs, 90.5% of them agreed or strongly agreed with the statement. These findings suggest that VR technology could be a valuable component to add to traditional hands-on training workshops in dairy farms.

Key Words: immersive educational technology, milking training, Spanish-speaking dairy worker

Reproduction 1: Posters

2690 Impact of cumulus cells quantity on cumulus-oocyte complexes on recovery and embryo production rates after IFIOT in cattle. A. B. Covre^{*1}, J. E. V. Chaves², A. C. C. V. Nicolás³, H. B. S. Amaral³, L. Mardones¹, J. F. W. Spricigo², M. A. N. Dode⁴, and J. O. Carvalho¹, ¹*Federal University of Espírito Santo, Alegre, Espírito Santo, Brazil*, ²*Federal University of Goiás, Goiânia, Goiás, Brazil*, ³*University of Brasília, Brasília, Distrito Federal, Brazil*, ⁴*Embrapa Genetic Resources and Biotechnology, Brasília, Distrito Federal, Brazil*.

This study aimed to evaluate whether immature cumulus-oocyte complexes (COC) with higher or lower amounts of cumulus cells (CC) injected into a dominant follicle (DF) influence the oocyte recovery rate 19 h after IFIOT and blastocyst production. For this, COC with higher (HL-CC, n = 220) or lower (LL-CC, n = 260) CC, from slaughterhouse ovaries, were injected into a DF ≥ 10 mm of 25 cows previously synchronized in 3 distinct replicates. With the injection, COC with higher (IVP HL-CC, n = 98) or lower (IVP LL-CC, n = 139) CC were used for in vitro maturation, serving as the control groups. The COC were measured before the maturation systems. After 19 h of injection, the COC were recovered via ovum pick-up (OPU) and sent to the laboratory for in vitro fertilization and culture. On d 2 (D2) and d 7 (D7) of culture, cleavage and embryonic rates were assessed. On D7, the embryos were fixed and evaluated for cell count using Hoechst staining. For the measurement of the COC, the HL-CC exhibited a greater measurement ($1,956.4 \pm 44.07 \mu\text{m}^3$) compared with the LL-CC ($1,089.9 \pm 49.45 \mu\text{m}^3$). The average oocyte recovery rate after OPU was 34.3% (75/220) for the HL-CC and 42.7% (110/260) for the LL-CC, being similar ($P = 0.08$). On D2, the cleavage rates were similar for the ML-CC (50.6%^a; n = 38/75) and LL-CC (66%^a; n = 66/103), with lower cleavage rates than the IVP ML-CC (82.6%^b; n = 81/98) and IVP LL-CC (78.4%^b; n = 109/139). On the D7, IVP HL-CC, 35 blastocysts (35.7%) were obtained from 98 COC, with 170 ± 10.9 cells, whereas in the IVP LL-CC, 51 blastocysts (36.7%) were obtained from 139 COC, with 167.5 ± 3.1 cells. In the IFIOT groups, 14 (18.7%) and 24 (23.3%) blastocysts were obtained from 73 and 103 COC in the HL-CC and LL-CC, respectively, with cell counts of 176.9 ± 8.3 and 180.6 ± 16.8 . Embryo numbers were similar between IVP groups using the same maturation system. Cell numbers were comparable across groups. The IFIOT found as a valid oocyte maturation model but yields lower blastocyst rates than IVP. Within IFIOT, HL-CC and LL-CC showed similar blastocyst development.

Key Words: intrafollicular immature oocyte transfer, cumulus-oocyte complex, embryo

2691 Effect of timing of administration of 325 mg of recombinant bovine somatotropin on the fertility of IVF Holstein heifer recipient. L. M. Rebeis^{1,2}, P. S. Baruselli², H. Harden¹, P. Ross³, P. Medellin⁴, B. Medellin⁴, M. Fosado³, J. F. Moreno³, and V. E. Gomez-Leon^{*1}, ¹*Kansas State University, Manhattan, KS*, ²*University of São Paulo, São Paulo, SP, Brazil*, ³*ST Genetics, Navasota, TX*, ⁴*APM Dairy Service, Syracuse, KS*.

Aim was to evaluate the effects of administering 325 mg of recombinant bovine somatotropin (BST) at different moments of a fixed-time embryo transfer (ET) protocol in heifers. Holstein recipients (n = 1,638, ~12 m old, first service only) from 2 farms were randomized to a 2 × 2 factorial design: BST on the day of starting the synchronization protocol (SYNCd, yes = 830 vs. no = 808), and BST on the day of the ET (ETd,

yes = 822 vs. no = 816). All heifers were submitted to a fixed-time ET synchronization protocol: d 8, CIDR insert; d 3, CIDR withdrawal, and 500 µg of cloprostenol; d 0, 86 µg of gonadorelin. The d 6, ultrasound to identify heifers with a corpus luteum (CL). The ET was conducted on d 7, using grade 1 fresh IVF blastocyst. Pregnancy diagnoses were conducted on d 36, 66, 85, and 120, and generalized linear models were used to assess the fertility effect of BST on SYNCd, ETd, and their interaction. Heifers treated with BST on SYNCd had ~18% greater pregnancy/ET at d 36 (41.1%) when compared with those untreated (34.8%; $P = 0.01$). Likewise, pregnancy loss between d 36 and 66 was reduced by ~50% when recipients were treated with BST on the SYNCd (14.7%) compared with untreated counterparts (22.4%; $P = 0.003$). This effect was maintained until d 120 (BST on SYNCd preg loss d 36–120 = 21.2% vs. untreated = 30.3%; $P = 0.02$). Moreover, BST on the ETd and the interaction of BST SYNCd by ETd had no effect on fertility in the current study. To assess the effect of BST on physiologic parameters, a subset of recipients was used to measure the largest follicle d 3 (n = 267), the CL volume on d 6 (n = 223), and the embryo crown-rump length on d 36 (n = 256). Data were analyzed with GLIMMIX (SAS Enterprise Guide v8.4). Although BST did not change the largest follicle size, the CL volume tended ($P = 0.08$) to be larger, and the embryo size was greater in heifers treated with BST on the SYNCd than in untreated ones (13.3 vs. 14.0 ± 0.2 mm; $P = 0.001$). In conclusion, administering 325 mg of rbST at the beginning of a fixed-time ET protocol but not on the day of ET enhanced the fertility of Holstein heifer recipients. Such effect may be mediated by corpus luteum and embryo development. Acknowledgment: Union Agener, ST Genetics, APM Dairy Service, & USDA/NIFA Hatch Multistate project 1014097

Key Words: embryo transfer, pregnancy loss, dairy heifer

2692 Effect of short dry period on reproductive performance of Holstein dairy cows. M. Ahmed¹, A. Riaz¹, N. Ahmad¹, M. N. Haque¹, H. Rahman¹, M. Yaseen¹, T. Ashraf¹, M. Abdullah¹, A. Hassan¹, A. Husnain¹, Z. Hassan¹, and Z. Sarwar^{*2}, ¹*University of Veterinary and Animal Science, Lahore, Punjab, Pakistan*, ²*University of Florida, Gainesville, FL*.

This experiment aimed to investigate the effects of dry period length on postpartum energy balance (EB) and reproductive performance, as well as to assess the mRNA abundance of key genes (FSHR, LHCGR, STAR, CYP19A, CYP11A, IGF1, IGFBP5) in granulosa cells of Holstein cows. Holstein cows were blocked on 207 ± 3 d of gestation by previous lactation milk yield, and within the block, they were randomly assigned to either a traditional dry period of 60 d (TDP; n = 22), or a short dry period of 30 d (SDP; n = 22). Cows in TDP received a dry diet for 30 d followed by an acidogenic diet until calving, whereas SDP cows were fed an acidogenic diet immediately after drying off. All cows had similar postpartum diets. Intake of DM and milk yield were recorded for 21 DIM, and milk components were assessed weekly. Cows were inseminated on detected estrus between 50 and 70 DIM and after that noninseminated cows were timed AI using G6G protocol. The granulosa cells were harvested at 7 d after AI. The reproduction of cows was followed by 300 DIM. Data were analyzed using mixed effects models (MIXED, GLIMMIX, and PHREG procedures) in SAS. The production and reproduction performance are presented in Table 1. The experiment revealed that SDP cows produced 8.4 kg/d less milk but had improved postpartum EB than TDP cows. However, the mRNA abundance of

Table 1 (Abstr. 2692). Effect of dry period length on performance in Holstein cows

Parameter	Treatment ¹			<i>P</i> -value
	TDP	SDP	SEM	
Prepartum				
DMI, kg/d	11.27	10.59	0.78	0.54
EB, Mcal/d	2.02	2.15	1.03	0.93
Postpartum				
DMI, kg/dDMI, kg/d	15.54	16.38	1.03	0.46
EB, Mcal/d	−3.40	3.26	1.15	< 0.01
Milk yield, kg/d	23.62	15.21	1.90	< 0.01
Pregnancy by 300 DIM, %	77.66	90.86	10.30	0.27
Days open				
Median (95% CI)	125 (89–204)	85 (60–164)	—	
Adjusted HR (95% CI)	Referent	2.46 (1.07–5.67)	—	0.03

¹Treatment = effect of dry period length 60 d vs 30 d.

genes did not differ between treatments. Cows in SDP treatment were bred faster and had fewer open days than TDP cows.

Key Words: dry period length, reproduction, follicular dynamic

2693 High pregnancy rates after an integrated nutritional and reproductive preventive and interventional program for early postpartum reproductive programming of Holstein dairy cows. J. M. Maldonado-Estrada*, S. Rios-Munera, M. F. Reina-Pulgarin, and S. Arango-Arcila, *Universidad de Antioquia, Medellin, Antioquia, Colombia.*

Background: A dairy cow management program called The PRIME RL La Montaña was established for dairy cows bred at the high-altitude tropical dairy farm from the University of Antioquia's dairy facility, located in the highland tropics of Antioquia Region (Colombia) at 2400 m altitude. The program aimed to prevent clinical and subclinical hypocalcemia, monitor postpartum uterine disease up to 35 DIM, promote early postpartum ovarian reactivation, and start FTAI if no standing heat at 45 DIM. Objective: To evaluate the effect of PRIME RL on pregnancy rates. Methods. Cows were bred under permanent grazing in Kikuyu grass (*Cenchrus clandestinus*) and added with concentrated feed and *Tithonia diversifolia* silage supplementation based on individual requirements. All cows (n = 51) received a transition cow diet and were assessed after calving for PUD diagnosis and treatment, ovarian reactivation, AI if exhibiting standing heat between 40 and 45 DIM, or a modified FTAI protocol at 45 DIM. The UdeA-Synch protocol consisted of d 1 GnRH1, d 7 GnRH2, and PGF2alpha, followed by AI at d 9–11 only if exhibiting estrous signs, and GnRH3 immediately after AI. Corpus luteum color Doppler assessment was performed at 17 to 23 d after AI for ruling out nonpregnant cows, followed by pregnancy diagnosis and confirmation at 35 and 60 d after AI, respectively. Cows were assessed by rectal palpation and ultrasound diagnosis using Mindray Z5 equipment with a 5.0 MHz probe from March to December 2024, comprising the wet (March to May) and rainy (June to December) seasons. Pregnancy rates and services per conception were compared before and after PRIME RL. Data were evaluated by descriptive statistics. Results. Probable nonpregnant cows (lack of CL color Doppler signal) were 10%. The pregnancy rates at 35 and 60 d after AI were 90% and 62%, respectively, resulting in a 28% embryo loss rate. According to dairy control data, no cows suffered from metabolic disorders using an automated MilkoScan FT3 assessment of raw milk. Different cows suffered from retained placental

membranes, clinical metritis (n = 1 each), or subclinical endometritis (n = 2), as evidenced by ultrasound assessment. No clinical or subclinical hypocalcemia was detected. Services per conception (S/C) were 3.6 (30.3% fertility) and 1.6 (63.2% fertility) before and after implementing the PRIME RL program. Conclusions: Integrative management through the PRIME RL program resulted in a 62% pregnancy rate 60 d after AI.

Key Words: dairy cow, fixed-time artificial insemination, pregnancy rate

2694 Could ultrasonographic backfat measurement replace body condition score assessment as a routine practice in dairy cows? J. M. Rojo-Bedoya¹, H. J. Correa-Cardona², L. G. Placio-Baena¹, J. G. Maldonado-Estrada^{*1}, and S. Arango-Arcila¹, *¹Universidad de Antioquia, Medellin, Antioquia, Colombia, ²Universidad Nacional de Colombia, Medellin, Antioquia, Colombia.*

Background: A BCS assessment by visual inspection/palpation in dairy cows is subjective. Ultrasound imaging of back fat thickness (BFT mm) is a noninvasive technique allowing quantitative subcutaneous fat reserve assessment. Objective: Compare the correlation between ultrasonographic BFT measures with BCS assessment during the transition period and lactation in dairy Holstein cows bred in a high-altitude tropical farm. Methods: The study analyzed BCS, BFT, parity, live weight, the projected net lactation energy requirement for maintenance (NEL Mcal/d), projected DMI kg/d, and DIM from prepartum (n = 10), 45 DIM (n = 18), and all 3-thirds of lactation (n = 51). The BFT measurements were made at 5 anatomical points: P1 (between L5 and L6), P2 (between the sacral crest and the beginning of the coxal tuberosity), P3 (between the coxal and the iliac tuberosities, 5 cm above the greater trochanter), P4 (above the insertion of the superficial gluteal muscle), P5 (between C4 and C5, aside tail insertion). The anechoic image taken over the loin and left haunch between the superficial fascia (subcutaneous) and the deep (muscular) fascia was considered as fat, using a Mindray Z5 Vet ultrasound scanner with a linear probe ranging from 3.5 to 7.5 MHz. For each point of evaluation, triplicate data of each image were recorded for data analysis. Results were compared with the conventional BCS evaluation. Simple regression evaluation between dorsal fat and BCS values was used to evaluate the data, and completely randomized unbalanced ANOVA was applied because the cows evaluated each time differed. Results: No significant difference in BFT (mm) was found between P1 (22.83 ± 4.14), P2 (22.72 ± 4.06), P3 (23.14 ± 4.37), P4 (23.30 ± 5.06), and P5 (22.72 ± 4.06). Anatomical points were the fixed factor,

and cows were the random factor. Spearman ($r = 0.59$) and Kendall ($r = 45$) correlation coefficients were applied between BCS and BFT up to 48 DIM. Likewise, Bland-Altman analysis was used, and a polynomial regression $R^2 = 0.58$ was found between these 2 variables. The BCS was 3.58 ± 0.53 , 3.00 ± 0.69 , and 2.74 ± 0.58 . The BFT (mm) was 23.95 ± 1.87 , 22.21 ± 5.35 , and 22.84 ± 4.02 . Parity was 2.9 ± 1.60 , 3.2 ± 1.52 , and 3.2 ± 1.52 . Weight (kg) was 653.40 ± 42.79 , 636.11 ± 66.93 , and 605.35 ± 59.36 . The projected NE_L (Mcal/d) requirement for maintenance and DMI (kg/d) were $(14.78 \pm 0.72, 11.75 \pm 0.88)$ and $(12.57 \pm 0.74, 17.10 \pm 2.14)$ for the first 2 groups, whereas DIM ($25.78 \pm 14.36, 77.04 \pm 68.41$) and L/d production ($33.44 \pm 5.85, 30.24 \pm 7.21$) for the last 2 groups. Ultrasound images captured with the probe at a frequency of 5 MHz provided the highest quality images, supporting P1 and P3 as the most suitable anatomical points to measure BFT. Conclusions: BFT assessment in dairy cows at P1 and P3 can be routinely used to estimate subcutaneous body fat reserves in dairy cows.

Key Words: body condition score, dairy cow, ultrasound

2695 Effect of treatment with GnRH at the time of AI on fertility outcomes in lactating dairy cows detected in estrus using tail chalking or automated monitoring devices. V. A. B. Primo^{*1}, T. D. Gonzalez², C. J. Middleton¹, M. B. Ugarte Marin¹, J. A. Gibaud¹, R. C. Chebel^{1,3}, and R. S. Bisinotto¹, ¹Department of Large Animal Clinical Sciences, D. H. Barron Reproductive and Perinatal Biology Research Program, University of Florida, Gainesville, FL, ²Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA, ³Department of Animal Sciences, University of Florida, Gainesville, FL.

Objective was to evaluate the effect of a GnRH injection at the time of AI on pregnancy outcomes in lactating dairy cows inseminated following estrus detection. Cows from a single farm were observed for signs of estrus based on removal of tail chalk ($n = 492$) or activity and rumination patterns characterized by automated monitoring devices (AMD; $n = 1,134$) after the first AI postpartum. Within estrus detection strategy, cows were randomly assigned to receive 86 μ g of gonadorelin acetate i.m. on the day of AI ($n = 814$) or remain as untreated controls ($n = 812$). Pregnancy was diagnosed by transrectal ultrasonography on d 33 ± 3 and 67 ± 7 after AI. Binary variables were analyzed by multivariable logistic regression using the GLIMMIX procedure of SAS. Pregnancy per AI (P/AI) on d 33 was affected ($P = 0.03$) by the interaction between treatment and estrus detection method, as GnRH increased P/AI in cows detected in estrus using AMD (GnRH = 52.2 vs. Control = 43.9%; $P < 0.01$) but not tail chalking (GnRH = 43.2 vs. Control = 47.0%; $P = 0.40$). Moreover, P/AI was affected ($P < 0.01$) by the interaction between treatment and service number, as GnRH increased P/AI in cows receiving the third or later AI (GnRH = 45.8 vs. Control = 36.9%; $P = 0.02$), but not in those receiving the second AI postpartum (GnRH = 49.7 vs. Control = 54.3%; $P = 0.19$). Contrary to the initial hypothesis, treatment with GnRH increased P/AI in cows with intense estrus behavior defined using a proprietary algorithm (Heat Index; $HI \geq 90$; GnRH = 60.0 vs. Control = 46.0%; $P < 0.001$) but not among those with weak estrus alerts ($HI < 90$; GnRH = 49.2 vs. Control = 48.7%; $P = 0.92$). Treatment with GnRH did not affect ($P = 0.16$) the proportion of pregnancy loss between d 33 and 67 after AI (GnRH = 3.6 vs. Control = 5.8%). Pregnancy loss was greater ($P = 0.04$) for cows inseminated in response to an AMD alert compared with removal of tail chalk (7.0 vs. 3.0%). Increased P/AI in response to GnRH in cows monitored using AMD suggests a potential effect on the synchrony between AI and ovulation.

Key Words: reproduction, fertility, estrus detection

2696 Efficacy of accelerometers to detect cows in estrus after not conceiving to artificial insemination. L. Zapata¹, J. Bartolome¹, and P. Melendez^{*2}, ¹National University of La Pampa, General Pico, La Pampa, Argentina, ²City University of Hong Kong, Hong Kong, China.

The objective of this observational study was to describe the proportion of cows not detected in estrus by accelerometers after not conceiving to AI and, in cows not detected in estrus, to report clinical findings at ultrasonography. The study was conducted in a confined dairy herd located northwest of Buenos Aires province, Argentina, milking 1,300 cows 3 times a day, with a rolling herd average of 12,657 L. During February and June 2025, 1,093 cows were timed AI after using a synchronization of ovulation protocol. Return to estrus was monitored by rumination and activity accelerometers (CowManagerSensor, Agis Automatisering, Harmelen, the Netherlands) placed on the ear tag. The system was capable of assessing real-time eating, rumination, resting, high activity (estrus), ear-surface temperature, and heat alerts. Three hundred sixty-four cows were pregnant (pregnancy per AI, 33.3%). In multiparous, the pregnancy per AI was 32.8%, and in primiparous, 34.0% ($P > 0.05$). Within season, pregnancy per AI was 19.7% in summer, and 38.3% in winter ($P \leq 0.05$). Within parity and season, the pregnancy per AI was summer multiparous 19.6%, summer primiparous 19.8% ($P > 0.05$), winter multiparous 38.0%, and winter primiparous 38.8% ($P > 0.05$). Six hundred fifty-two cows were open and detected in estrus, and 77 were not detected in estrus but resulted open at ultrasonography. High activity was detected in cows in estrus compared with 3 d before and after the estrus. Among those cows detected open, 41.3% had a CL and received PGF, and 58.7% did not have a CL and received GnRH. Differences in treatment type (PGF vs. GnRH) within parity were not significant (multiparous PGF 37.8% vs. primiparous PGF 46.7%; $P = 0.24$). In summer, multiparous PGF cows were 50%, and primiparous PGF was 20%, whereas in winter, multiparous PGF cows were 32.3%, and primiparous PGF was 52% ($P = 0.13$). In conclusion, the accelerometers were highly efficient in detecting cows not conceiving to AI that repeated estrus (89.4%); however, there were still cows that did not conceive and were not detected in estrus by the system (10.6%), influenced by the breeding season and parity that need to be clinically examined as soon as possible by accurate diagnosis methods to improve the fertility of the herd.

Key Words: accelerometer, heat detection, fertility

2697 Effect of length of stay in postpartum pen and genomic net merit on presence of active corpus luteum, milk yield, and reproductive performance of Holstein dairy cows. L. Prada Gutierrez^{*1}, G. Schuenemann¹, S. M. Bellinzoni¹, J. Lakritz², A. Garcia Guerra³, and G. M. Schuenemann¹, ¹Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, ²Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH, ³Department of Animal Sciences, The Ohio State University, Columbus, OH.

The objective was to assess the effect of length of stay in postpartum pen (LSPP) and genomic lifetime net merit (NMS) on presence of active corpus luteum (CL), milk yield (MY), and pregnancy status of dairy cows. A total of 817 multiparous Holstein cows from 2 Ohio dairy herds were enrolled in monthly cohorts of 20 to 36 cows at 21 ± 3 d before expected calving date. At calving, cows were randomly assigned into 1 of 3 LSPP: 7 ($n = 260$), 14 ($n = 283$), or 21 DIM ($n = 274$). Once each LSPP group was completed, lactating cows were moved into the high lactation group. Cows were fed a TMR formulated to meet nutritional requirements for prepartum, postpartum, and high lactation diets. Serum

Table 1 (Abstr. 2697).

Item	Length of stay in postpartum pen relative to calving, d			P-value
	7	14	21	
Lactation, n	2.76 ± 0.05	2.84 ± 0.05	2.84 ± 0.05	0.49
CL, %	33.20 ± 3.06	38.66 ± 2.94	36.88 ± 2.98	0.42
DIM at first AI, d	72.70 ± 0.57	73.75 ± 0.55	73.72 ± 0.55	0.44
PAIF, %	41.84 ± 3.16	34.90 ± 3.02	31.87 ± 3.05	0.06
MY, kg/d	44.74 ± 0.90	44.65 ± 0.90	45.95 ± 0.90	0.54
Milk fat, kg/d	2.22 ± 0.08	2.15 ± 0.08	2.17 ± 0.09	0.80
Milk protein, kg/d	1.46 ± 0.04	1.42 ± 0.04	1.44 ± 0.04	0.79
ECM, kg/d	51.99 ± 1.08	50.49 ± 1.17	52.46 ± 1.25	0.47

progesterone (≥ 1 ng/mL) was assessed at 28 ± 3 DIM to determine presence of an active CL. The MY (including milk fat and milk protein) and ECM at first DHI were assessed. For first AI, all cows were subjected to double-Ovsynch with timed-AI. The PAI was determined for first (PAIF), first 2 services combined (PAIC), and up to 300 DIM. Lifetime NM\$ was obtained for a subset of cows ($n = 406$). Data were analyzed using MIXED, PHREG, or GLIMMIX procedures of SAS. Farm was included as a random effect. The LSM $\pm$ SEM were reported. Overall CL, MY, milk fat, milk protein, and ECM did not differ among LSPP groups. Although PAIF tended to differ, AHR (95% CI) for pregnancy up to 300 DIM ($P = 0.018$) were 1.31 (1.08–1.58; $P = 0.005$) and 1.09 (0.91–1.32; $P = 0.35$) for 7 and 14 LSPP, respectively (21 LSPP as reference). Furthermore, cows with NM\$ >450 had a 32.57% higher PAIC ($P = 0.003$) compared with cows with NM\$ ≤ 450 . Although CL and MY (or components) did not differ by groups, these findings show that 7 LSPP cows had improved reproductive performance (Table 1).

Key Words: transition dairy cow, lifetime net merit, pregnancy

2698 Effects of retained placenta on conception rate and pregnancy loss in tropical dairy herds. K. R. Oliveira^{*1,2}, T. E. da Silva², J. H. C. Costa², T. M. Ferreira³, C. E. C. Consentini⁴, R. Sartori⁵, A. L. Silva¹, and P. P. Rotta¹, ¹Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil, ²University of Vermont, Burlington, VT, ³Alta Genetics do Brasil, Uberaba, Minas Gerais, Brazil, ⁴GlobalGen Vet Science, Jaboticabal, São Paulo, Brazil, ⁵ESALQ, University of São Paulo, Piracicaba, São Paulo, Brazil.

Retained fetal membranes negatively impact embryonic development due to uterine inflammation and infection, reducing conception rate (CR) and increasing pregnancy loss (PL) by impairing placental establishment. This study evaluated RP effects on CR and PL in tropical dairy herds. Data from January 2021 to December 2023 were obtained from “Concept Plus Dairy” (Alta Genetics do Brasil) across 8 Brazilian states and analyzed in RStudio. Farms with launching errors, absence of RP records, missing production system information, and reproductive management frequency were removed. After cleaning, 307 farms with Holstein and Holstein $\times$ Gyr (HG) cows housed in compost barns (68.04%), free stalls (19.45%), or semiintensive systems (12.50%) remained, totaling 262,187 AI services for CR and 61,529 AI pregnancies for PL analysis. Binomial models using “glmmTMB” included farm as a random effect and RP interaction with breed, parity, AI order, semen type, and AI condition (timed AI or estrus). Data included 166,348 Holsteins, 42,367 3/4 HG, 37,835 7/8 HG, and 15,637 1/2 HG cows, totaling 98,791 primiparous and 163,396 multiparous cows. Cows were inseminated with conventional (236,242) or sexed semen (25,945), with 72,011 AI on estrus and 190,176 timed AI, distributed in first (124,679),

second (82,783), or third or more AI (54,725). The CR was significantly affected by RP, breed, AI condition, semen type, parity, and AI order ($P < 0.0001$). The RP interacted with AI order and AI condition for CR, and with AI order for PL. The HG (1/2) cows had higher CR and lower PL, differing from Holsteins but not from 3/4 HG and 7/8 HG. Sexed semen reduced conception odds by 12% (OR = 0.88) compared with conventional semen. Parity and AI order negatively correlated with CR and positively with PL. The AI order interacted with RP for PL and CR: without RP, first and second AI had similar PL but differed from third or more and from each other in CR. With RP, cows had similar PL across AI services. RP reduced CR by 13% (OR = 0.87) and increased PL (OR = 1.41), significantly impacting herd profitability. Retained placenta has a detrimental effect on reproductive performance, reducing conception rates and increasing pregnancy loss, highlighting the importance of preventive strategies to improve fertility outcomes in dairy herds.

Key Words: tropical dairy system, crossbreed cow

2699 Optimization of rapid resynch program for lactating dairy cows using estradiol-based protocols. J. P. Andrade^{*1}, P. L. J. Monteiro², N. M. P. M. Caparelli³, M. G. Costa³, T. B. Almeida³, G. C. S. Pontes⁴, J. P. P. Oliveira⁴, A. B. Prata⁵, C. E. Consentini⁵, M. R. B. Mello³, and M. C. Wiltbank⁶, ¹West Texas A&M University, Canyon, TX, ²University of Florida, Gainesville, FL, ³Federal Rural University of Rio de Janeiro, Seropedica, RJ, Brazil, ⁴Rehagro Agribusiness Consulting, Belo Horizonte, MG, Brazil, ⁵GlobalGen Vet Sciences, Jaboticabal, SP, Brazil, ⁶University of Wisconsin, Madison, WI.

This study describes the effect of an estradiol-based protocol to optimize the ReBreed21 (RB21) program for rapid resynch in lactating dairy cows. Cows were blocked by first timed service (artificial insemination [TAI, $n = 490$] or embryo transfer [TET, $n = 239$]), parity (primiparous [$n = 302$] or multiparous [$n = 427$]), location (Southeast Brazil, farms A, B, or C), and randomized into 1 of 2 strategies for the subsequent services Resynch ($n = 363$) or Control ($n = 366$). On d 14 after induced ovulation (i.e., 14 d after TAI or 7 d after TET), Resynch cows received an intravaginal P4 insert (2 g) for 5 d, were treated with 1 mg of estradiol cypionate (d 19), and milk sampled at d 20 evening for P4 detection using a commercial on-farm milk rapid test. Low P4 cows received 8 μ g of Buserelin on d 20 (evening) and were TAI on d 21 morning (RB21). After the first service, control cows underwent daily estrus detection and AI (EAI). Pregnancy diagnoses were performed at 35 d of gestation, and nonpregnant cows that had not been inseminated in both groups were resynchronized using an estradiol-based protocol for subsequent TAI or TET according to the manager’s criteria. In the first service, pregnancy per AI (P/AI) was evaluated using a generalized linear model, including the block factors and treatment (Resynch or Control). Interaction between treatment and blocks was offered to the model. P/AI for subsequent service (ReBreed21, EAI, TAI, or TET) were analyzed, including farm and parity as fixed effects. P/AI in the first service for cows in Resynch was lower (33.3%; $P = 0.01$) than in Control (43.0%). At d 26 after the first service, 60.2% (242) of nonpregnant cows had already been inseminated in Resynch+RB21 compared with Control using EAI (16.8% [209]; $P < 0.01$). Compared with the Control, nonpregnant cows reached the subsequent service 11 d earlier in Resynch+RB21 (40 d vs. 29 d, $P < 0.01$). The P/AI was similar in the subsequent services (RB21 33.1% [142]; EAI 41.2% [73]; TAI 27.6% [47]; and TET 33.8% [148]; $P = 0.20$). Although RB21 achieved an acceptable P/AI, the early resynchronization treatment decreased P/AI during the first service. Further refinements are needed to enhance the RB21’s effectiveness.

Key Words: reinsemination

2700 Impact of body condition score on conception in dairy heifers. A. L. Ollinger^{*1}, A. Ahmadzadeh², S. Crawford¹, and J. A. Spencer¹, ¹Texas A & M University, College Station, TX, ²University of Idaho, Moscow, ID.

Dairy heifer reproductive success impacts herd sustainability and producer profitability. This study aimed to investigate the impacts of heifer BCS on number of services per conception (S/C) and days from birth to confirmation of pregnancy. Seventy-two Holstein dairy heifers from 2 Central Texas dairy farms were enrolled in this study. Body condition scores were determined for each dairy heifer ($n = 72$) at the time of entry into the breeding pen by 2 individuals and averaged. Body condition scores were evaluated on a 1 to 5 scale with 0.25-unit increments, and any heifers with ≥ 0.75 -unit difference between evaluator scores were discussed and agreed upon to at least a 0.5-unit difference between scores. Results were analyzed using R Statistical Software v. 4.2.3., with significance declared at $P < 0.05$ and tendency at $P \leq 0.1$. Statistical analysis using a negative binomial regression model ($P = 0.96$) observed no significance between BCS and number of S/C. A classification and regression tree analysis were used to predict the expected number of services to conception among varying BCS. In our sample, we found BCS values of 3.3 to 4.1 had a greater S/C (1.8 inseminations) than cows with BCS below this range (1.4 inseminations), but there was not enough data to say that this effect is proven across the greater population. An increase in BCS decreased the number of days from birth to confirmation of pregnancy by 69.33 d ($P < 0.001$) and entry into the breeding pen to confirmed pregnancy by 18.96 d ($P = 0.005$). These results indicate that BCS may influence days to pregnancy impacting age at calving. Therefore, BCS may be a valuable measurement for heifer reproductive management. Because of the limited sample size and possible confounding variables, the full explanation of BCS warrants further study.

Key Words: dairy heifer, body condition score, reproduction

2701 Long-term changes in endometrial morphology and functionality in dairy cows with metritis. M. H. Rashid^{*1}, A. Martelo-Pereira¹, F. N. S. Pereira¹, M. C. Oliveira Costa¹, E. F. Santos¹, J. A. Sexton¹, M. Cicarelli¹, L. B. Williams¹, M. Binelli², M. S. Waqas¹, E. B. de Oliveira³, and C. C. Figueiredo¹, ¹Washington State University, Pullman, WA, ²University of Florida, Gainesville, FL, ³Zoetis Inc., Parsippany, NJ.

Metritis affects ~20% of postpartum dairy cows and is a major risk factor for subsequent subfertility due to its carryover effect. The study aimed to assess long-term effects of metritis on endometrial morphology and functionality in dairy cows. Postpartum Holstein cows ($n = 140$) at the University of Idaho Dairy Center were enrolled in this cohort study. Vaginal discharge (VD) was evaluated visually within 12 DIM for the diagnosis of metritis. Cows with metritis ($n = 11$; MET) had fetid, watery, reddish/brownish VD and received antimicrobials. Cows without metritis (clear to purulent VD; $n = 11$; NoMET) of similar parity and DIM were paired as controls. Double Ovsynch was used to synchronize ovulation starting at 35 DIM, and ovulation was confirmed by ovarian ultrasonography. Cows were not inseminated. Six days after ovulation, endometrial biopsy and cytology were collected. Endometrial morphology/functionality was evaluated through H&E staining of histologic sections and light microscopy, transcriptomics (mRNA sequencing), and metabolomics (untargeted gas chromatography). For transcriptomic data (biopsy), gene expression between MET and NoMET was compared using DESeq2. Metabolomic analysis (cytology) was conducted using Partial least square discriminant analysis and PERMANOVA. Numerically, histologic assessment (biopsy) showed a

reduced number of endometrial glands in MET versus NoMET (mean, 13 glands/10 $\times$ field vs. 15 glands/10 $\times$ field) and a greater percentage of cows with endometrial fibrosis in MET versus NoMET (63% vs. 44%). On transcriptomic analysis, 17 genes were differently expressed ($FDR \leq 0.10$) between MET and NoMET. Briefly, genes related to cell differentiation and regeneration, including FOS, FOSB, EGR1–3, and SPPI1, were upregulated in MET, whereas genes related to immune function, including AOC1, FCMR, and ICOS, were upregulated in the NoMET group. Enrichment analysis showed that TNF signaling pathway was upregulated ($P = 0.05$) in MET group. No differences in uterine metabolome (cytology) were associated with metritis. Our data suggest that metritic cows sustain chronic endometrial damage which is manifested in transcriptomic changes but not translated to the uterine metabolome.

Key Words: metabolome, uterus, transcriptome

2702 Efficacy of automated activity monitoring technology at detecting cyclicity postpartum—A metaanalysis. A. M. L. Madureira^{*1} and S. Borchardt², ¹Michigan State University, East Lansing, MI, ²Free University Berlin, Berlin, Germany.

Estrous expression in early lactation is a robust predictor for fertility in lactating dairy cows. It is uncertain whether estrous expression, detected by automated activity monitors (AAM), can be used as a proxy for resumption of cyclicity. The objective of this study was to evaluate AAM systems to identify cyclicity of cows in early lactation. A systematic review of the literature and a metaanalytical assessment was performed. We identified 4 studies with 7 experimental groups with information on estrous expression and ovulatory status in early lactation including 2,198 cows. MedCalc was used to analyze the data, applying a Freeman-Tukey transformation to compute the weighted summary proportion under both fixed and random effects models. A fixed or a random effects meta-analysis was used based on the heterogeneity among the experimental groups. Blood samples in all experiments were collected 2 times or 3 times 14 d apart for progesterone (P4) analysis. Resumption of cyclicity was considered when P4 concentration was ≥ 1 ng/mL on any collection day. Cows were considered anovular when P4 concentration was < 1 ng/mL on all collection days. Cows were classified as true positive: P4 ≥ 1 ng/mL and at least 1 estrus alert; true negative: P4 < 1 ng/mL and no estrus alerts. Overall, there were 81.2% (1,785/2,198) of ovulatory cows in early lactation. Overall, there were 63.3% (1,393/2,198) of cows detected in estrus by AAM in early lactation. The pooled proportion of true positive cows was 88.1% (95% CI: 84.9–90.9). The pooled proportion of true negative cows was 35.3% (95% CI: 26.3–44.7). Substantial heterogeneity existed among the experimental groups regarding true positive ($I^2 = 63.7\%$; $P = 0.011$) and true negative cows ($I^2 = 85.0\%$; $P = 0.001$). In summary, most cows that exhibited estrus early in lactation also ovulated. However, the majority of cows that did not show estrus in early lactation still ovulated. Therefore, the absence of estrous activity in early lactation is not a reliable predictor of anovulation.

Key Words: estrous expression, automated activity monitor, resumption of cyclicity

2703 Impact of estrous expression and interval from estrus alert to synchronization protocol on pregnancy per AI in lactating dairy cows. S. Borchardt¹, T. A. Burnett², and A. M. L. Madureira^{*3}, ¹Free University Berlin, Berlin, Germany, ²University of Guelph, Ridgetown, ON, Canada, ³Michigan State University, East Lansing, MI.

The aim of this retrospective observational study was to evaluate the effect of estrous expression before the beginning of a synchronization

protocol and at the moment of AI on pregnancy per AI (P/AI). A total of 2,842 lactating Holstein cows from 1 commercial herd were enrolled. In yr 1, cows underwent a Double-Ovsynch (DO) protocol for first AI ($n = 1,536$) and produced a total 305-d milk yield of 13,421 kg, whereas in yr 2, cows followed a presynch-ovsynchrony (PO) protocol ($n = 1,306$) and produced 13,868 kg over 305 d. Cows were classified as primiparous or multiparous and continuously monitored using ear-tag accelerometers. Estrus alerts were identified by the system, and intervals from the last alert before synchronizations were categorized as short (SI; 7–14 d) or long (LI; 14–30 d). The total number of alerts from calving to AI was also recorded, with cows categorized as 0, 1, or ≥ 2 alerts. Pregnancy was confirmed by ultrasonography on d 32 post AI. A logistic regression model assessed associations between estrous expression and P/AI, including estrus at AI, parity, number of alerts, and estrus interval classification. Cow was a random effect, and protocol was a fixed effect. Primiparous cows were more likely to express estrus at AI than multiparous cows (OR 1.79; 95% CI 1.52–2.10). Estrus at AI improved P/AI (OR 1.55; 95% CI 1.32–1.82). For cows showing estrus, P/AI was $65.4\% \pm 2.4\%$ under DO and $51.1\% \pm 4.0\%$ under PO. Without estrus, P/AI was $54.4\% \pm 1.8\%$ and $44.5\% \pm 3.5\%$ for DO and PO, respectively. Multiparous cows with ≥ 2 alerts had higher P/AI (OR 1.65; 95% CI 1.10–2.48) than those with 1 alert (OR 1.32; 95% CI 0.98–1.78) or none. No significant difference was found in primiparous cows. LI cows had lower P/AI (OR 0.62; 95% CI 0.51–0.76) than SI cows. Under DO, SI cows had higher P/AI (OR 1.79; 95% CI 1.47–2.17) than LI cows, and under PO, SI cows also showed greater P/AI (OR 1.67; 95% CI 1.30–2.14). In conclusion, cows with SI before synchronization and estrus at AI had improved P/AI. Continuous monitoring can optimize protocol timing and reduce blanket treatments through selective management.

Key Words: estrous expression, synchronization protocol, fertility

2704 Circular RNAs are selectively secreted into conditioned medium by bovine preimplantation embryos. Y. Fan^{*1}, K. C. Pavani², A. Van Soom², and L. Peelman¹, ¹*Department of Veterinary and Biosciences, Faculty of Veterinary Medicine, Ghent University, Merelbeke-Melle, Belgium*, ²*Department of Internal Medicine, Reproduction and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke-Melle, Belgium*.

Circular RNAs (circRNAs) are closed-loop, mostly noncoding RNAs that play functional roles in various biological processes, including the regulation of preimplantation embryo development. They can be secreted by cells into biofluids as mediators of intercellular communication. However, whether preimplantation embryos secrete circRNAs into their surrounding environment remains unknown. In this study, we investigated this possibility using in vitro-produced bovine embryos. Seven bovine blastocyst-enriched circRNAs (top 1% of read counts in sequencing data, NCBI SRA ID: PRJNA1083451), including circGNPTAB(5,6,7,8), circHIPK3(1), circSUC2(7,8,9), circARID1A(4), circCAMSAP1(3,4), circSAMD4A(2), and circTPST1(2), were selected to validate their presence in conditioned medium. To confirm their circular structure, RT-PCR and Sanger sequencing were performed, successfully validating their back-splicing junctions. A total of 300 bovine preimplantation embryos were cultured in groups of 25 in 50 μ L of synthetic oviduct fluid medium until d 8 postinsemination. The conditioned medium (pooled to 600 μ L) and 15 blastocysts were collected for RNA isolation, followed by circRNA detection using RT-qPCR. The presence of 7 circRNAs, along with U6 snRNA as a positive control, was assessed. Because the aim was to determine their presence or absence, no replicates were included, and no statistical analyses were performed. All 7 circRNAs and U6 snRNA were detected in blastocysts

(Cq values <35). However, in the conditioned medium samples, only 4 circRNAs (circGNPTAB(5,6,7,8), circHIPK3(1), circSUC2(7,8,9), and circTPST1(2)), along with U6 snRNA (Cq value around 26), were detected, exhibiting Cq values between 30 and 35. The remaining 3 circRNAs were undetectable (Cq values >40). These findings demonstrate that bovine preimplantation embryos can selectively secrete circRNAs into their conditioned medium, suggesting a potential role for circRNAs in early embryonic communication and biomarker discovery.

Key Words: circular RNA, bovine embryo, conditioned medium

2705 Is the pregnancy loss in high-yielding dairy cows during the second month of gestation initiated by luteal failure or embryonic death? R. Shaked¹, L. Lifshitz¹, M. C. Wiltbank², and U. Moalem^{*1}, ¹*Department of Ruminant Science, Volcani Institute, Rishon LeZion, Israel*, ²*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI*.

In recent years, it was found that ~15% of pregnancy losses (PL) in high-yielding dairy cows occurred during the second month of pregnancy. This study was focused on the mechanism leading to PL from d 28 to 63 of pregnancy. We hypothesized that PL occurs in some cows due to luteal failure, and in other cows due to conceptus failure before luteal regression; therefore, the objectives were to determine whether the primary initiator of pregnancy loss is inadequate corpus luteum maintenance or embryo death, and if exogenous progesterone (P_4) supplementation will reduce the PL incidence. For this aim, 116 high-yielding dairy cows from the experimental dairy farm of Volcani Institute (Israel) that had a confirmed pregnancy on d 28 post AI were divided into control ($n = 70$) and treatment ($n = 46$) groups. Twice or thrice weekly, blood samples were collected for P_4 and pregnancy-associated glycoprotein (PAG) determination, and rectal ultrasonography was performed weekly. In the treatment group, 2 internal drug-releasing inserts (CIDR) of P_4 were inserted and changed every 7 d until d 63 of pregnancy. The data were analyzed using the PROX MIXED of SAS. The mean P_4 concentration from 28 to 63 d was greater by 1.44 ng/mL (15%) in the treatment than in control cows (9.4 and 10.8 ng/mL, respectively; $P < 0.001$). The blood PAG started to increase from d 19 and peaked at 33 d post AI, then decreased to 2 OD at 63 d, with no differences between groups in this pattern. Ten out of 70 cows (14.3%) from the control and 8 out of 46 cows (17.4%) from the treatment group ($P = 0.77$) lost their pregnancy between 35 to 51 d post AI. According to the PAG and P_4 timing and patterns in the blood of the cows that lost pregnancy, 10 out of 18 cows (55.5%) lost their pregnancy probably due to CL regression, 6 cows probably due to embryo death (33.3%), and 2 cows (11.1%) for unknown reasons. In conclusion, the PL incidence in this study was within the range that was reported previously, with ~55% of these losses initiated by regression of the CL and ~33% initiated by the death of the conceptus; that is, luteolysis occurred before a decrease in PAG, or PAG decreased, but luteolysis did not occur, respectively. Moreover, exogenous P_4 supplementation did not reduce the PL incidence during the second month of pregnancy.

Key Words: dairy cow, pregnancy loss

2706 The association between energy metabolism-related variables and conception rate at first service in Holstein cows. P. Melendez^{*1}, G. Gonzalez², G. Lastra-Duran², and P. Pinedo³, ¹*Jockey Club College of Veterinary Medicine & Life Sciences, City University of Hong Kong, Hong Kong, China*, ²*Dairy Granja Palestina, Torreón, Coahuila, Mexico*, ³*Department of Animal Sciences, Colorado State University, Fort Collins, CO*.

This study examined the relationship between energy metabolism–related variables and conception rate at first service (CRFS) in Holstein cows. The research was conducted on a large dairy farm in Coahuila, Mexico, milking 3×, and 1,553 cows with an ME 305 d milk production of 12,800 kg. The voluntary waiting period was 60 d, and the farm used a Double-Ovsynch protocol for the first breeding. The dataset considered 1,056 lactations (419 primiparous, 637 multiparous) from 2024. The records were extracted from the software Dairy Com 305. The inclusion criteria for the analysis were only cows that completed the synchronization protocol and were timed AI. The first step was a univariate analysis for the following independent variables: BCS at caving, BCS losses, SCK (blood BHB ≥ 1.2 mmol/L 5–8 DIM), milk yield at wk 8 pp, parity, breeding season, and other diseases (retained fetal membranes/metritis). The second step was a multivariable analysis using a logistic regression model. In the univariate analysis, each variable was significant with CRFS. However, in the multivariable model, BCS at calving and other diseases were not significant when both SCK and BCS losses remained in the model. Furthermore, BCS at calving and other diseases were associated with SCK and BCS losses. Cows with BCS at calving ≥ 3.75 were 5.55 times more likely to experience BCS losses ≥ 0.75 units from calving to first breeding than cows with BCS ≤ 3.5 . Cows with BCS at calving ≤ 3.5 were 0.45 times as likely to develop SCK as cows with BCS ≥ 3.75 . Additionally, cows with other diseases were 4.42 times more likely to develop SCK than healthy cows. The best fitting logistic regression model for CRFS considered as significant variables: breeding season (Cool vs. Hot; OR = 1.25, 95% CI = 1.09–2.24); milk yield at wk 8 pp (for extra 1 kg of milk; OR = 1.03, 95% CI = 1.005–1.050); parity (Prim vs. Mult; OR = 1.60, 95% CI = 1.21–2.12); SCK (No vs. Yes, OR = 1.66, 95% CI = 1.23–3.42); BCS loss (≤ 0.5 vs. ≥ 0.75 , OR = 1.51, 95% CI = 2.09–3.08). It is concluded that SCK and BCS losses were important variables associated with CRFS that were also confounded by BCS at calving and other diseases.

Key Words: BCS, ketosis, fertility

2707 Effect of presynchronization with prostaglandin $F_{2\alpha}$ 2 days before initiation of 2 CIDR-Synch protocols in Holstein heifers. W. M. Brown^{*1}, I. M. R. Leão², K. Skillin², P. Crave¹, M. R. Lauber¹, J.

P. N. Martins², and P. M. Fricke¹, ¹*Department of Animal and Dairy Science, University of Wisconsin–Madison, Madison, WI*, ²*School of Veterinary Medicine, University of Wisconsin–Madison, Madison, WI*.

Our objective was to assess the effect of presynchronization with PGF_{2 α} on progesterone (P4) concentration at the first GnRH treatment of 2 CIDR-Synch protocols and resulting pregnancies per AI (P/AI) after timed AI (TAI) in nulliparous Holstein heifers. Heifers (n = 501) from 2 Wisconsin dairy farms were randomized to a 2 × 2 factorial design to test the main effects of protocol (5D, n = 236 vs. 6D, n = 265), and presynchronization with 25 mg PGF_{2 α} 2 d before initiation of the protocols (–PG, n = 249; +PG, n = 252). All heifers received GnRH/CIDR on d 0; PGF_{2 α} on d 5 and 6; and GnRH on d 8. The CIDR inserts were removed either on d 5 in which TAI occurred at GnRH treatment on d 8 (5D), or on d 6 in which TAI occurred 8 h after GnRH treatment on d 8 (6D). All heifers were inseminated with sexed semen. Blood samples were collected at the first GnRH treatment to measure serum P4 concentration. Variables were analyzed by logistic or linear regression (R studio 4.4.1). The model included protocol, presynchronization, their interactions as fixed effects, and farm as random effects. There was no presynchronization by protocol interaction on P/AI at 32 or 60 d after TAI. Overall, P4 concentration at the first GnRH treatment of the protocols was less ($P < 0.01$) for +PG heifers than for –PG heifers (1.5 ± 1.9 ng/mL vs. 5.1 ± 3.6 ng/mL). There was a tendency for presynchronized heifers to have more ($P = 0.09$) P/AI at 32 d after TAI (50.6% vs. 44.6%), but presynchronization with PGF_{2 α} did not affect ($P = 0.14$) P/AI at 60 d after TAI (47.4% vs. 42.6% for +PG vs. –PG heifers, respectively). Although timing of CIDR removal is confounded with timing of AI, P/AI did not differ between protocols at 32 d after TAI (49.2% vs. 46.2% for 5D vs. 6D heifers, respectively [$P = 0.26$]) or at 60 d after TAI (46.6% vs. 43.6% for 5 d vs. 6 d heifers, respectively [$P = 0.25$]). We conclude that although presynchronization with PGF_{2 α} decreased P4 at the first GnRH treatment of both CIDR-Synch protocols, presynchronization only tended to increase P/AI at 32 d but not at 60 d after TAI in Holstein heifers. Supported by WI Experiment Station Hatch project WIS04012.

Key Words: dairy heifer, sexed semen, timed artificial insemination

Ruminant Nutrition 3: General—Posters

2708 Supplementation with a blend of essential oils, tannins and bioflavonoids in dairy cows. I: Implications for milk production and feed conversion efficiency. J. G. Minutti^{*1}, N. Cavassa³, J. I. Paez³, B. Suarez¹, G. Fernandez¹, J. L. Repetto², and A. Santana¹, ¹Facultad de Veterinaria, San José, Uruguay, ²Facultat de Veterinària, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain, ³Facultad de Ciencias Agrarias UDE, Montevideo, Uruguay.

The study evaluated the effects of a blend of essential oils, tannins, and bioflavonoids (EOTB, ANAVRIN, Vetos Europe SAGL, Switzerland) on DMI, milk production, and feed efficiency (FCE) in dairy cows fed fresh forage (FF) and a partial mixed ration (PMR). Sixteen multiparous Holstein × Jersey cows (57 ± 31 DIM) in a crossover design were assigned to: (1) Control (PMR at 70% of potential DMI with 8 h unrestricted FF access) and (2) EOTB (Control diet + 3 g EOTB/cow/d). The 85 d experiment included 2 periods (30-d adaptation, 12 d data collection). Cows were weighed on d 0, 42, and 85. For 5 d, individual DMI of PMR and FF (19% DM, 23% CP, 45% NDF) was calculated as difference between offered and refused. Milk yield (kg) was recorded over 10 milkings, 4 samples being analyzed for composition using midinfrared. Data were analyzed by the PROC MIXED of SAS (fixed effects: treatment, period, and their interaction; random effect: cows) with AR(1) covariance structure for repeated measures. FCE increased by 7% in EOTB cows, mainly due to a 4% reduction in DMI. Supplemented cows required ~100 g less DMI per kilogram of 3.5% SCM. No effects were observed on milk yield or composition, except a slight increase in fat percent. Our results demonstrated an improvement in nutrient utilization and efficiency in cows receiving EOTB (Table 1).

Key Words: phytogetic additive, milk composition, productive performance

2709 Feeding behavior and dry matter intake associated with feeding 2 concentrations of an *Asparagopsis armata* containing product. B. L. Gast^{*1}, L. R. Brown¹, M. L. Simonds¹, A. M. Searing Hettel², J. Keller³, P. Russo³, J. A. Pempek⁴, and J. P. Boerman¹, ¹Department of Animal Sciences, Purdue University, West Lafayette, IN, ²Department of Food Science, Purdue University, West Lafayette,

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Supplementation of *Asparagopsis armata* (A-A) has been reported to reduce enteric methane emission from cattle; however, less is known about how seaweed products impact feeding behavior and diet palatability. We hypothesized that cows fed a higher concentration of A-A will have a lower DMI and altered feeding behavior compared with cows fed a diet with no A-A or diet with lower concentration of A-A. Nine nonlactating, nongestating Holstein dairy cows averaging 48 ± 11 mo of age and 690 ± 83 kg of BW at study initiation were enrolled in a replicated 3 × 3 Latin square with each period lasting 21 d. Diets included a control diet with no A-A (CON), low A-A diet (LA-A; bromoform concentration 6 mg/g DM), and high A-A diet (HA-A; bromoform concentration 9 mg/g DM). Immediately after feeding, cows were observed for 15 min and scored every minute using a feeding behavior ethogram. Intake was assessed after 1 h and after 23 h access to feed each day of the study. Data were analyzed using SAS v 9.4 with the fixed effects of square, day of treatment, and treatment, and the random effect of cow and period. There were no differences between treatments for 1 h intake ($P = 0.19$); however, daily intake was 0.6 kg/d lower for HA-A compared with LA-A ($P < 0.05$). There were no differences for feeding behavior ethogram results for the proportion of time spent eating during the first 15 min of offered feed ($P = 0.11$). There was a treatment by day of treatment effect for 1 h intake ($P < 0.05$) with cows consuming 0.9 kg less feed in the first hour new feed is offered compared with the second day feed is offered. Overall, A-A did not affect the palatability of the diet to the extent that it reduced feed intake relative to control, but the higher dose of A-A did have reduced daily intake compared with the lower dose of A-A. Regardless of diet, cows consumed a significantly lower amount of feed on the first day a novel diet was presented.

Key Words: palatability, seaweed, feeding ethogram

2710 Impacts of peripartum comorbid development of fatty liver and hyperketonemia on performance and health. G. F. Praisler¹, T. L. France¹, T. F. Wallace¹, R. Fukumori^{1,2}, U. Arshad¹, S.

Table 1 (Abstr. 2708). Intake, production, and feed efficiency of dairy cows fed fresh forage (FF) plus partial mixed ration (PMR) supplemented with a blend of essential oils, tannins, and bioflavonoids (EOTB)

Item ¹	Treatment			P-value		
	Control	EOTB	SEM	Treatment	Period	Treatment × period
DMI, kg/d						
PMR	13.9	13.8	0.080	0.370	<0.010	0.020
FF	9.2	8.5	0.290	0.020	<0.010	0.610
Total	23.2	22.3	0.360	0.020	<0.010	0.320
Total DMI, % BW	4.1	4.0	0.090	<0.010	<0.010	0.190
Milk yield, kg/d	29.5	29.8	1.660	0.790	0.840	0.650
Fat, %	4.0	4.2	0.120	0.030	<0.010	0.840
Protein, %	3.8	3.8	0.090	0.620	<0.010	0.250
Lactose, %	4.9	4.9	0.050	0.870	0.130	0.210
SCM 3.5%	32.1	33.0	1.600	0.323	0.231	0.564
BUN, mg/dL	18.0	18.2	0.370	0.530	<0.010	<0.010
FCE	1.3	1.4	0.060	0.030	<0.010	0.350

¹SCM = solid corrected milk at 3.5% = (0.4324 × milk yield kg) + (16.218 × fat kg); FCE = feed efficiency = SCM (kg/d)/DMI (kg/d).

J. Kendall¹, and H. M. White^{*1}, ¹University of Wisconsin–Madison, Madison, WI, ²Rakuno Gakuen University, Ebetsu, Hokkaido, Japan.

Peripartum metabolic disorders, fatty liver disorder (FLD), and hyperketonemia (HYK), may have additive negative effects when occurring as comorbidities (Comorb). The objective of this study was to examine the impacts of comorbid onset of FLD and HYK in peripartum cows. Multiparous Holstein cows ($n = 28$) were blocked and enrolled in study at -28 d relative to calving (DRTC) and in study until 56 DRTC in a tiestall facility. Dry matter intake and milk yield were recorded daily. Body weights, blood, and liver samples were taken across the peripartum period and BW change (Δ BW) calculated from -21 to 28 DRTC. Liver triglyceride (LvTG) concentrations were quantified by Folch extraction on a DM % basis. Cows were retrospectively categorized into 3 groups (GRP): NonFLD, FLD (LvTG $\geq 13.9\%$ DM), and Comorb (LvTG $\geq 13.9\%$ DM and plasma BHB ≥ 1.2 mM within 14 DRTC). Pre- and postpartum data were analyzed separately in a mixed model (SAS 9.4) with fixed effect of GRP, DRTC, GRP $\times$ DRTC, and the random effect of cow within block. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. Preplanned contrasts of FLD_{all} (FLD&Comorb vs. NonFLD) and Comorb_{only} (Comorb vs. NonFLD&FLD) were examined if main effects $P \leq 0.15$. Prepartum DMI tended to be less in FLD and Comorb (FLD_{all} $P = 0.08$, Comorb_{only} $P = 0.07$), whereas postpartum DMI was less (Comorb_{only} $P = 0.02$) for Comorb_{only}. The Comorb cows had greater (Comorb_{only} $P = 0.02$) prepartum BW, tended to have greater ($P = 0.06$) Δ BW, and FLD_{all} had greater ($P = 0.02$) Δ BW. Milk yield did not differ by GRP, but Comorb_{only} had decreased ($P \leq 0.04$) milk protein and SNF %. The Comorb_{only} had greater ($P \leq 0.05$) postpartum glucose and LBP, and also experienced decreased ($P \leq 0.03$) prepartum AST and SAA. Postpartum insulin was decreased ($P = 0.001$) in FLD_{all}. Postpartum AST:ALT was greater ($P = 0.0003$) in Comorb_{only}. Pre- and postpartum plasma fatty acids were greater ($P \leq 0.04$) for FLD_{all}. Although this study was not powered to explore impacts on milk yield, these data suggest that there may be associations between comorbid development of HYK and FLD impacts on DMI and health indicators.

Key Words: transition cow, metabolic health

2711 Effect of essential oils and organic acids on feeding behavior in dairy cows during peak lactation. L. S. Lopes, R. M. da Rosa, W. S. Rosa, T. C. da Silva, M. T. Vieira, U. S. Londero, E. R. Komninou, V. R. Rabassa, L. Marins, R. Silveira, A. C. T. Rodrigues, M. L. dos Santos, F. A. B. Del Pino, and M. N. Corrêa*, *Federal University of Pelotas, Pelotas, Rio Grande do Sul, Brazil.*

Essential oils (EOs) are natural compounds with therapeutic properties that can improve intestinal health and the productive performance of animals. However, their impact on feeding behavior and feed intake in dairy cows during peak lactation, especially when combined with organic acids, is still underexplored. This study evaluated the effects of a supplement containing EOs and organic acids on milk production, feed intake, and feeding behavior in multiparous Holstein cows during peak lactation. Forty cows were divided into 2 groups: treatment (TG, $n = 20$), receiving 3 g/d of a product containing clove, orange, and cashew EOs, along with fumaric, sorbic, and citric acids (Ruminatus, Safeeds Nutrição Animal, Paraná, Brazil), and control (CG, $n = 20$), without supplementation. All cows received the same diet (TMR) and management in a compost bedded-pack barn. Feed intake was monitored using electronic feeders (Ponta Agro, Minas Gerais, Brazil), feeding behavior was recorded with electronic collars (CowMed, Santa Maria, Brazil), and milk production was measured using the ALPRO system (DeLaval, Sweden). Statistical analysis was performed using JMP Pro 14, with 1-way ANOVA and Tukey's test ($P < 0.05$). No significant differences

were observed in DMI (GC: 21.6 ± 0.22 kg/d vs. TG: 22.08 ± 0.22 kg/d, $P = 0.14$) or milk production (GC: 35.62 ± 0.32 kg/d vs. TG: 36.36 ± 0.32 kg/d, $P = 0.11$). However, the TG group showed a higher feeding time (125.5 ± 1.63 min/d vs. 140.3 ± 1.75 min/d, $P < 0.001$), more visits to the feeders (7.7 ± 0.07 vs. 9.2 ± 0.07 , $P < 0.001$), less rumination time (498 ± 2.56 min/d vs. 488 ± 3.14 min/d, $P = 0.01$), and more idle time (455.6 ± 2.26 min/d vs. 475 ± 2.67 min/d, $P < 0.001$). Supplementation with EOs and organic acids did not affect milk production or DMI but resulted in increased feeding activity and decreased rumination time.

Key Words: digestion, feeding behavior, nutritional efficiency

2712 A multispecies postbiotic improved the performance and health of dairy cows compared with yeast-based products on a commercial dairy in hot climate. J. Kaufman*, C. Nett, and R. Lall, *RD LifeSciences LLC, Largo, FL.*

The study objective was to assess a multispecies fungal and bacterial postbiotic compared with a combination of yeast culture additives on the production and health of commercial dairy cows in a hot climate. A year-over-year longitudinal field study was conducted with 13,200 lactating Holstein cows in equal months from June 2022 until October 2023. The average temperature-humidity index was 78.0 in 2022 and 77.6 in 2023. Cows were in a barn with evaporative cooling. Cows were fed Yea-Sacc and Integral A+ products (PC) before June 2022 until the first week of July 2023. After, cows transitioned to a multispecies fungal and bacterial postbiotic, SPEC 7^T at 5 g/cow/d (SPEC7; RD Lifesciences, Largo, FL), with July as an adaptation period. Milk yield, milk components, and pen intake were assessed for 16 wk. To satisfy potential confounding effects from the year-over-year study, mean differences were also compared against the reference month of June for each year. Data were analyzed using a PROC GLIMMIX in SAS v9.4. The SPEC7 did not impact pen DMI for lactating cows. The SPEC7 decreased ($P < 0.01$) fecal starch content from 3.6% to consistently $<2\%$ each month. The SPEC7 increased ($P < 0.01$) milk and ECM yields by 1.3 and 2.4 kg/d compared with PC, respectively. Likewise, SPEC7 increased ($P < 0.01$) milk fat and lactose percentages by 0.21 and 0.11 units, respectively, and tended to increase ($P = 0.10$) milk protein percentages by 0.06 units compared with PC. The SPEC7 increased ($P < 0.01$) the efficiency for ECM yield by 5.6% compared with PC (1.88 vs. 1.78). When going into a hotter climate, SPEC7 reduced ($P < 0.01$) the drop in ECM yield difference and efficiency difference by 0.60 kg/d and 0.07 units compared with PC. The SPEC7 decreased ($P < 0.01$) SCC on average per month by 23,100 cfu/mL and decreased ($P < 0.01$) SCC overall by 92,500 cfu/mL in 2023 compared with no change from PC in 2022. The return on investment for SPEC7 was 12:1. Overall, SPEC7 supported improved milk production, feed efficiency, and health likely from increased digestibility and immune support to cows in a hot climate.

Key Words: postbiotic, feed additive, heat stress

2713 Effect of a combination of encapsulated and nonencapsulated oregano oil on milk yield and milk components of dairy cows. A. Beckmann*, T. Borchardt¹, K. Fenske², and H. Westendarp², ¹Dostofarm GmbH, Westerstede, Germany, ²Department of Animal Nutrition, University of Applied Sciences, Osnabrück, Germany.

Oregano essential oil (OEO) is becoming increasingly popular in dairy cow feeding due to its rumen modulating effects, which can have a positive impact on milk performance and metabolic health. The aim of this study was to investigate the effect of a combination of rumen-available and rumen-protected oregano oil on milk yield and milk components of

multiparous lactating dairy cows. One hundred twenty Holstein cows (185 DIM) were divided into 2 groups (control group and trial group) according to parity, lactation stage, milk yield, and SCC. The trial was conducted over 9 mo, during which the trial group received 420 g/animal/d OEO (DOSTO Ruminant Bi-active, Dostofarm GmbH, Westerstede, Germany). This supplement was pelleted into the concentrate feed and administered during milking in the automatic milking system. Analysis of variance was carried out using the SAS software package (SAS Institute Inc., Cary, NC, version 9.4) for the robot data (milk yield) and SPSS (IBM, version 28) for the milk control data (milk components). To compare the mean values, the differences of the LSM were determined. The significance level was $P < 0.05$. The multiparous cows that received the OEO supplement had on average a numerically higher milk yield (41.3 kg vs. 40.0 kg; $P = 0.24$). In the first 100 d of lactation, milk yield was on average 1.60 kg higher in trial group compared with control group ($P = 0.31$). Milk fat and milk protein content were numerically higher in the trial group compared with the control group in almost all of the 8 conducted milk controls. In the overall analysis, the milk fat and milk protein content were on average 0.07% (3.93% vs. 3.99%; $P = 0.34$) and 0.05% (3.50% vs. 3.45%) higher ($P = 0.16$) in the trial group compared with the control group. The SCC in the milk were not affected in this study ($P = 0.97$). The supplementation of a combination of rumen-available and rumen-protected OEO to lactating dairy cows led to a numerical increase in milk yield and milk protein and milk fat. There is a need for further research in this field, especially regarding the effects on the rumen microbiome and metabolic health.

Key Words: oregano, cow, milk yield

2714 Effect of a phytogenic feed additive, autolyzed yeast, and organic minerals on dairy cows around the induction of ruminal acidosis. J. T. R. Carvalho¹, R. B. Silva², A. J. C. Silva¹, M. A. Tiengo¹, C. D. S. Oliveira^{1,2}, J. O. Martins¹, B. M. S. Borges³, M. L. Vasconcelos³, O. F. Zacaroni³, R. A. N. Pereira⁴, C. S. Cortinhas⁵, V. V. Carvalho⁵, and M. N. Pereira^{*1}, ¹Universidade Federal de Lavras, Lavras, MG, Brazil, ²Better Nature Research Center, Lavras, MG, Brazil, ³Universidade Federal de Goiás, Goiânia, GO, Brazil, ⁴Empresa de Pesquisa Agropecuária de Minas Gerais, Lavras, MG, Brazil, ⁵dsm-firmenich, São Paulo, SP, Brazil.

A phytogenic feed additive (PHY, Digestarom, DSM) and autolyzed yeast plus organic minerals can reduce ruminal acidosis in dairy cows subjected to slug-feeding. Fifteen Holstein cows (40.0 ± 6.9 kg/d, 103 ± 54 DIM, 607 ± 81 kg) were fed a sequence of treatments, control (CTL), PHY (0.01% DM), and YOM (PHY + 0.075% DM of yeast + 0.15% DM of organic sources of S, Cu, Mn, Zn, Se) in 3 × 3 Latin squares with 35-d periods (22-d adaptations). Cows were milked 3 ×/d and individually fed on TMR (17.0% CP, 31.2% starch). During d 29 and 32 of each period, feed was removed from each cow at 1900 h and cows were fed ad libitum on d 30 and 33 at 0700 h to induce slug-feeding. Data obtained daily from d 23 to 35 were analyzed as repeated measures with MIXED procedure of SAS. The model had the effects of square, cow (square), period, treatment, day, and the interaction between treatment and day ($P \geq 0.33$ for all variables). Feed restriction on d 29 and 32 induced spikes in DMI and in the duration of the first daily meal on d 30 and 33 and acute depressions in milk yield, BW, and ruminal pH ($P < 0.01$ for day). Cows fed CTL had lower DMI than cows fed PHY and YOM (22.9, 23.5, 23.9 kg/d; $P < 0.02$). Milk yield did not differ (33.0 kg/d; $P = 0.40$), but ECM was lower on CTL than on PHY and YOM (31.0 kg/d vs. 31.9 kg/d; $P < 0.03$). Milk fat concentration was lower on CTL than on PHY and YOM (3.39% vs. 3.49%; $P < 0.01$). Preformed, mixed, and de novo fatty acids (g/100 g milk and g/d) were increased

by both PHY and YOM ($P < 0.05$), suggesting that the phytogenic feed additive drove the positive response in milk fat. Ruminal pH measured at 30-min intervals, fermentation profile, and microbial yield did not differ ($P > 0.16$). The total-tract digestibility of DM and OM tended to be greater on YOM than PHY ($P < 0.09$). There was a tendency ($P = 0.10$) for reduced gut permeability on PHY than CTL, evaluated by lower plasma Cr concentration 4 h after pulse dosing Cr-EDTA into the rumen, but serum concentration of LPS-binding protein did not differ ($P > 0.33$). Cows fed PHY and YOM had greater DMI, ECM, and fat yield than cows fed CTL.

Key Words: phytogenic feed additive, ruminal acidosis, yeast

2715 Effect of a blend of essential oils on milk fatty acid profile and plasma metabolites in Jersey cows transitioning from confinement to grazing. M. A. Arshad*, K. V. Almeida, D. C. Reyes, A. L. Konopoka, M. A. Rahman, M. M. H. Khandakar, R. A. Menezes, and A. F. Brito, *Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, NH.*

Essential oils (EO) have been used to modify ruminal fermentation, which may influence the milk fatty acid (FA) profile and blood constituents. We aimed to investigate the effect of supplementing a blend of EO (Agolin NATURU) on milk FA and plasma metabolites in dairy cows transitioning from winter (i.e., confinement) to grazing season. Twenty-two (6 primiparous and 16 multiparous) organic-certified Jersey cows (83 ± 39 DIM and 468 ± 51 kg BW) were enrolled in a randomized complete block design and assigned to the following treatments: (1) no EO supplementation (control = CON) or (2) 1 g/d of EO. The experiment lasted 12 wk, with 2 wk for covariate measurements and 10 wk for treatment evaluation. Data and samples collected during wk 5 (confinement) and wk 10 (grazing) were used for statistical analyses. Cows in confinement received (DM basis) a diet with 60% grass-legume baleage and 40% concentrate, with 35% of baleage replaced by grazed herbage in the diet DM during grazing. Milk samples were collected for 2 consecutive milkings, pooled by cow based on morning and afternoon milk yields, and analyzed for FA using GC. Blood samples were collected 2–3 h after the morning and afternoon feedings, with pooled plasma analyzed for glucose and urea N. The proportion of milk total 16C FA was greater ($P < 0.04$) with feeding EO than CON (28.8 g/100 vs. 27.8 g/100). However, the milk proportions of 18:2 c9, t11, 18:3 c9, c12, c15, total <16C FA, total odd-chain FA, total branched-chain FA, total trans-18:1, total cis-18:1, and total 18C FA did not change between diets. Feeding EO tended ($P \leq 0.09$) to increase the milk proportions of 18:2 c9, c12 (2.10 vs. 2.26), total n-6 FA (2.49 g/100 g vs. 2.34 g/100 g), and total n-3 FA (0.67 g/100 vs. 0.65 g/100) compared with CON, and increased ($P < 0.04$) the n-6/n-3 ratio (3.66 g/100 g vs. 3.56 g/100 g). Treatment did not affect ($P \geq 0.57$) the plasma concentrations of glucose (mean = 60.5 mg/dL) and urea N (mean = 18.9 mg/dL). Overall, supplementing EO increased milk total 16C FA and the n-6/n-3 ratio, without affecting the concentrations of plasma glucose and urea N in Jersey cows.

Key Words: essential oil, milk fatty acid, plasma

2716 Effects of supplementing Animax+ on aflatoxin M₁ levels in dairy cow milk. M. Al-Qaisi*, A. F. Souad², H. H. Titi¹, M. Abujamieh¹, M. A. Abdel-Majeed¹, and A. Abdelqader¹, ¹Department of Animal Production, School of Agriculture, the University of Jordan, Amman, Jordan, ²Manaseer Industrial Complex, Amman, Jordan.

Aflatoxins can adversely affect milk production, milk quality, and cow health. Besides, it poses a significant risk to human health. Hence, identifying nutritional approaches aimed at mitigating the aflatoxin levels in dairy products is warranted. The objective of this study was to evaluate the effects of toxin binder (Animax+) on cow productivity and the levels of aflatoxin M₁ in milk in lactating dairy cows. Ten lactating Holstein cows (564 ± 19 kg BW; 110 ± 22 DIM; 2.7 ± 0.4 parity) were used in an experiment. Cows were randomly assigned to 1 of 2 diets: a control diet (CON; n = 5) or the control diet supplemented with 1 g Animax+/kg diet DM (n = 5). Cows were fed their respective diets for 14 d. After this prefeeding phase, they entered a 4-wk experimental period. Milk yield was recorded daily. Milk and blood samples were obtained once weekly. Effects of treatment, week, treatment × week were analyzed using MIXED procedure of SAS. Milk production tended to increase in Animax+-fed cows relative to CON cows (26%; $P = 0.10$) in wk 3 and 4. Milk composition was unaltered by dietary treatments ($P \geq 0.31$). Furthermore, circulating lipopolysaccharide-binding protein, serum amyloid A, haptoglobin, and albumin were similar between dietary treatments ($P \geq 0.31$). However, feeding Animax+ reduced aflatoxin M₁ levels (22%; $P < 0.01$). In summary, supplementing with 1 g of Animax+/kg diet DM tended to increase milk yield and blunted aflatoxin M₁ levels.

Key Words: aflatoxins, dairy cow, toxin binder

2717 Effect of a blend of organic acids and a surfactant on in vitro rumen fermentation parameters and in vivo lactation performance in dairy cows. S. M. Tondini¹, J. M. C. De Souza², H. Khelil-Arfa¹, and J. C. McCann², ¹*Archer Daniels Midland Company (ADM), Decatur, IL*, ²*Animal Sciences Department, University of Illinois at Urbana-Champaign, Urbana, IL*.

The objective was to investigate the effects of a dietary supplement consisting of organic acids and a surfactant (DC) on in vitro rumen fermentation and degradability parameters and animal performance in dairy cows. An in vitro system was utilized to test 1 of 2 treatments over a 24 h period: (1) CON; no supplementation and (2) DC supplemented at 25 mg/flask. The diet consisted of 30% haylage, 25% corn silage, 20% corn, 15% canola meal, and 10% soybean hulls. At 24 h, in vitro DM disappearance (IVDMD) and NDF disappearance (IVNDFD) were measured. Supplementation with DC increased ($P < 0.05$) IVDMD and IVNDFD by 3.2% and 2.9%, respectively. There was a tendency ($P = 0.07$) for propionate (mM) to be increased by 15% in DC compared with CON at 12 h. The in vivo experiment was conducted at Moulins de Kerollet in Arzal, France. Eighty Holstein-Friesian dairy cows (38 primiparous and 42 multiparous) averaging 80 DIM and 36 kg/d of milk yield (MY; kg/d) were blocked by parity, DIM, and MY and randomly assigned to 1 of 2 treatments: (1) CON; no supplementation and (2) DC supplemented at 40 g/cow/d for 90 d. The basal diet consisted of 55% corn silage, 20% grass silage, 15% hay, and 10% soybean meal. Data were analyzed using a mixed model with fixed effects of treatment, week, parity, treatment × week, and treatment × parity. Animal and block were random effects. Milk yield and ECM (kg/d) increased ($P < 0.01$; 1.3 and 1.1 kg/d, respectively) in cows supplemented with DC. Additionally, there was a treatment × parity interaction ($P < 0.01$), with DC supplemented multiparous cows having a 7% increase (+2.7 kg/d) in MY when compared with CON. Additionally, DC supplementation increased ($P < 0.01$) milk fat (kg/d) and milk protein (kg/d) by 2.8% and 2.7%, respectively. Overall, supplementation with organic acids and a surfactant improved lactation performance by increasing substrate degradability and propionate concentration.

Key Words: fiber degradability, organic acid, surfactant

2718 Effects of extending the close-up period of nulliparous heifers on dry matter intake, body weight, milk production, and blood metabolite concentrations. N. Nishizawa¹, R. Harada², and M. Oba^{3,4}, ¹*Dairy Technology Research Institute, The National Federation of Dairy Co-operative Associations (ZEN-RAKU-REN), Nishi-shirakawa, Fukushima, Japan*, ²*The National Federation of Dairy Co-operative Associations (ZEN-RAKU-REN), Shibuya, Tokyo, Japan*, ³*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada*, ⁴*Graduate School of Integrated Science for Life, Hiroshima University, Higashi-Hiroshima, Hiroshima, Japan*.

The objective of this study was to evaluate the effects of extending the close-up period of nulliparous heifers from 4 wk to 6 wk on BW changes, blood metabolite concentrations, and milk production. Nulliparous heifers were assigned to either a control group with a 4-wk close-up period (4W, n = 19) or an experimental group with a 6-wk close-up period (6W, n = 15). From 10 wk before the expected calving date, heifers were fed far-off diets (CP 13.5%, starch 15.0%, NDF 46.6% on a DM basis) until 4 or 6 wk before calving. From 4 or 6 wk before calving until calving, they were fed close-up diets (CP 15.2%, starch 20.3%, NDF 38.3%), and after calving, lactation diets (CP 16.3%, starch 25.1%, NDF 30.5%). Dry matter intake, BW, milk yield and composition, and blood metabolite concentration data were collected from 10 wk before calving to 12 wk after calving. There was a Week × Treatment interaction for DMI before calving ($P < 0.05$); it was higher ($P < 0.05$) for the 6W group compared with the 4W group at 6 wk (11.2 kg/d vs. 9.7 kg/d) and 5 wk (11.0 kg/d vs. 9.8 kg/d) before calving. Treatment did not affect DMI after calving (18.5 kg/d vs. 17.7 kg/d; 6W vs. 4W, respectively), BW change before calving (0.01 kg/d vs. -0.00 kg/d), BW change after calving (-0.49 kg/d vs. -0.47 kg/d), serum total protein (6.5 g/dL vs. 6.5 g/dL), urea nitrogen (12.7 mg/dL vs. 13.3 mg/dL), total cholesterol (118 mg/dL vs. 118 mg/dL), free fatty acids (0.39 mEq/L vs. 0.34 mEq/L), BHB (831 μmol/L vs. 704 μmol/L), and blood glucose (64.7 mg/dL vs. 65.2 mg/dL). However, the 6W group had higher milk yield (33.9 kg/d vs. 29.9 kg/d; $P < 0.05$), protein yield (1.00 kg/d vs. 0.92 kg/d; $P < 0.05$), lactose yield (1.56 kg/d vs. 1.39 kg/d; $P < 0.05$), and solid nonfat yield (2.84 kg/d vs. 2.56 kg/d; $P < 0.05$). These results suggest that extending the close-up period of nulliparous heifers from 4 to 6 wk may increase milk production after calving possibly due to greater intake of protein and starch before calving.

Key Words: nulliparous heifer, close-up period, milk production

2719 Effects of feeding a *Bacillus licheniformis* fermentation extract with amylase activity on lactation performance in mid-lactation Holstein cows. C. M. K. Bradley*, V. L. Daley, and O. N. Genter-Schroeder, *Purina Animal Nutrition LLC, Gray Summit, MO*.

The objective of this research was to evaluate the effects of 2 levels of *Bacillus licheniformis* fermentation extract (BLFE) with amylase activity on milk production and composition in midlactation Holstein cows. Multiparous cows (n = 96, averaging 45.3 ± 9.7 kg/d milk and 138 ± 50 DIM) were stratified by milk production and DIM and assigned to treatments in a completely randomized design for 6 wk. Cows were housed in freestalls and assigned to individual Calan doors where a grain mix containing their treatment (Control, 5 g/d BLFE, or 10 g/d BLFE) was incorporated in their TMR. During the feeding period, milk weights were recorded at each milking, and BW were electronically captured as cows left the milking parlor. Milk samples were collected twice a week at all 3 milkings and analyzed for components. Fecal score and fecal pH were recorded for samples collected from 20 cows/

treatment during the last week of the study. Cow was the experimental unit and effects of treatment, time, and treatment by time interaction were included in the mixed model with repeated measures. Covariate data were also tested in the model. There was no effect of treatment on DMI, BW, fecal score, or fecal pH ($P > 0.05$). BLFE inclusion at 5 g/d increased milk, protein, TS, ECM, and FCM yields by 2.35, 0.07, 0.28, 2.13, and 2.10 kg/d, respectively ($P < 0.05$) relative to control. BLFE inclusion at 10 g/d increased milk, TS, and FCM yields by 1.73, 0.22, and 1.80 kg/d, respectively ($P < 0.05$) and tended to increase fat and ECM yields by 0.07 and 1.74 kg/d, respectively ($P < 0.10$), relative to control, but was not different from 5 g/d BLFE ($P > 0.40$). In conclusion, including BLFE at 5 or 10 g/d supported similar increases in milk and component yields of midlactation dairy cows.

Key Words: *Bacillus licheniformis*, fermentation extract, amylase

2720 Impact of *Bacillus subtilis* 810 and *Bacillus licheniformis* 809 on milk production, composition, and efficiency: A metaanalysis. K. M. Morrill*, S. Lerner, and K. Bryan, *Novonosis, Milwaukee, WI*.

The objective of this metaanalysis was to review research evaluating the effects of probiotic containing *Bacillus subtilis* 810 and *Bacillus licheniformis* 809 on lactation performance. We hypothesized that milk production and milk production efficiency would be greater for cows receiving a 2-*Bacillus*-strain probiotic as compared with control fed cows. After screening against a set of criteria, 8 trials were included in the metaanalysis (464 lactating cows). The metaanalysis was conducted in EXCEL, pooled SD was calculated by taking a weighted average of the variances (square of the SD) from the 2 groups, adjusted for the sample size. The SE of the difference was calculated to represent the uncertainty or variability in the estimate of the difference between 2 means. Initial DIM ranged from 0 to 126, and days on product ranged from 60 to 112. All trials provided treatment animals with 9.6 billion cfu/head/d of a probiotic containing *B. subtilis* 810 and *B. licheniformis* 809. Four trials contained only multiparous cattle, whereas 4 trials contained both multiparous and primiparous cattle. The metaanalysis revealed an improvement in milk production ($P < 0.01$), ECM production ($P < 0.05$), feed efficiency ($P < 0.05$), protein yield ($P < 0.05$) and a decrease in MUN ($P < 0.01$). There was a trend for an improvement in milk lactose yield ($P = 0.08$). These results support the hypothesis that the combination *B. subtilis* 810 and *B. licheniformis* 809 supports milk production and milk production efficiency. These findings highlight the benefits of *B. subtilis* 810 and *B. licheniformis* 809 to support performance throughout the lactation of dairy cattle

Key Words: probiotic, efficiency of production, bacilli

2721 Performance of cows fed a blend of additives during the summer. R. Chesini¹, G. G. da Silva¹, C. S. Takiya^{2,1}, C. S. Cortinhas³, C. C. Dipold¹, V. V. Carvalho³, and F. P. Rennó^{*1}, ¹University of São Paulo, Pirassununga, São Paulo, Brazil, ²Universidade Tecnológica Federal do Paraná, Pato Branco, Paraná, Brazil, ³dsm-firmenich, São Paulo, São Paulo, Brazil.

The combination of phytogetic additives, yeast products, and selected minerals may provide a positive synergistic effect on productivity and body temperature control in cows. This study evaluated the effects of supplementing a blend of feed additives (BLD) on feed efficiency, milk composition, and vaginal temperature (VT) in dairy cows. Twenty-six Holstein cows (144 ± 86 DIM; 34.4 ± 26.7 kg/d milk yield) were blocked ($n = 13$) by DIM and milk yield and randomly assigned to 1

of 2 treatments: (1) control (CON), no feed additives or (2) BLD, 50 g/d of BLD incorporated into the concentrate mix. The additive used in this study contained a formulated blend of extracts and essential oils (82.8 mg/kg diet DM, Digestarom, dsm-firmenich, Switzerland), carbo-aminophosphochelates minerals (200 mg Zn cow/d and 10 mg Cr cow/d; dsm-firmenich, São Paulo), autolyzed (620 mg/kg diet DM) and live yeasts (124 mg/kg diet DM), and niacin (248 mg/kg diet DM, Rovimix Niacin, dsm-firmenich, Switzerland). The experiment lasted 11 wk, with the first 2 wk used for covariate data collection. Cows were individually fed a TMR and were milked twice daily. Milk samples were analyzed for solids using midinfrared spectroscopy. VT of cows was recorded every hour by a datalogger (Hobo TidbiT). Data were analyzed using the mixed procedure of SAS, with fixed effects of covariate, treatment, time, and their interactions, and a random effect of block. Average air temperature, relative humidity, and THI were $25.5 \pm 1.27^\circ\text{C}$, $65.8\% \pm 1.27\%$, and 73.7 ± 1.88 , respectively. DMI and milk yield were similar between treatments. FCM 3.5% (35.5 kg/d vs. 39.3 kg/d for CON and BLD, respectively) and ECM (35.9 kg/d vs. 39.5 kg/d for CON and BLD) yields were greater ($P \leq 0.03$) for BLD-supplemented cows. Milk fat production increased (1.29 kg/d vs. 1.43 kg/d for CON and BLD), and milk fat concentration tended to increase with BLD supplementation. Feed efficiency (FCM/DMI) was also greater ($P = 0.03$) in cows fed BLD compared with CON (1.20 kg/d vs. 1.34 kg/d for CON and BLD). VT was decreased ($P = 0.03$) with BLD (39.4°C vs. 39.2°C). The blend of additives improves feed efficiency and FCM yield, while reducing VT in dairy cows under high THI conditions.

Key Words: phytogetic additive, heat stress, organic mineral

2722 Effects of supplementing a *Bacillus*-based direct-fed microbial on milk production and composition of lactating dairy cows offered a low-starch total mixed ration. O. C. M. Queiros*, J. N. Jorgensen, A. C. S. Storm, and B. I. Cappelozza, *Novonosis, Lyngby, Zealand, Denmark*.

Microbial solutions, such as direct-fed microbials (DFM), have been fed to dairy cows to support their health and productive performance. However, the effects of DFM, more specifically of *Bacillus*-based, on performance of lactating dairy cows consuming a low-starch TMR are scarce in the literature. Hence, we evaluated the effects of feeding a *Bacillus*-based DFM on milk production and composition of lactating Holstein cows offered a 17.0% starch TMR. Seventy-six primiparous ($n = 24$) and multiparous ($n = 52$) lactating Holstein dairy cows (25 ± 3 DIM; initial BW 686 ± 82.8 kg) were blocked by parity, DIM, and previous milk yield (for multiparous only) to a TMR containing or not (CON; $n = 38$) *Bacillus licheniformis* 809 and *Bacillus subtilis* 810 (Bovacillus; Novonosis, Lyngby, Denmark; BAC; $n = 38$) for 12 wk. The TMR contained 45.4% concentrate and 54.6% forages (DM basis), and all cows had ad libitum access to water and the TMR. Throughout the study, DMI and milk yield were recorded daily, whereas milk samples were collected 4 times a week for determination of fat, protein, lactose, urea, and SCC. Based on the milk composition results, the fat and protein-corrected milk (FPCM) yield was calculated. Finally, income over feed cost (IOFC) was calculated by subtracting the input on milk yield and feed cost. All data were analyzed using the GLIMMIX procedure of SAS, using cow as the experimental unit. No treatment $\times$ week interaction was observed for any of the variables analyzed herein ($P \geq 0.20$). Mean DMI (25.0 kg vs. 25.7 kg), milk yield (39.2 kg/cow vs. 40.5 kg/cow), FPCM yield (40.2 kg/cow vs. 41.6 kg/cow), and IOFC were greater (11.33 USD/cow vs. 11.65 USD/cow; $P \leq 0.03$) for BAC, but no effects were observed on feed efficiency ($P \geq 0.44$). Milk solids yield (kg/d) was also greater ($P \leq 0.03$) for cows fed BAC (fat: 1.64 kg vs. 1.68 kg; protein: 1.33

kg vs. 1.38 kg; lactose: 1.81 kg vs. 1.87 kg, respectively), whereas no further differences were observed on milk composition or efficiency of N utilization ($P \geq 0.24$). Hence, supplementing a *Bacillus*-based DFM increased milk production, composition (kg/d), and the profitability of dairy cows offered a low-starch-based TMR. Nonetheless, additional work is warranted to understand the potential modes of action by which such results have been observed.

Key Words: *Bacillus*-based direct-fed microbial, dairy cow, income over feed cost

2723 Administration of a spice feed additive in mid lactating dairy cows: Effects on feeding behavior and intake, performance, and milk composition. L. Cattaneo¹, G. Desrousseaux^{*2}, F. Piccoli Cappelli¹, and E. Trevisi¹, ¹*Department of Animal Sciences, Food and Nutrition, Università Cattolica del Sacro Cuore, Piacenza, Italy*, ²*Phodé, Terssac, France*.

The aim of this study was to evaluate the effects of the supplementation of a spice feed additive in the diets of mid lactating high-producing dairy cows on health status, feeding behavior and feed intake, rumen pH, and milk yield and composition. Thirty-four mid lactating Holstein dairy cows (parity = 2.2 ± 1.0 ; milk yield = 43.1 ± 6.4 kg; DIM = 131.8 ± 47.6 ; BW = 632 ± 51 kg [mean $\pm$ SD]) were enrolled in the study. Cows were housed in the same freestall pen and received a typical lactation TMR of Northern Italy based on corn silage, alfalfa hay, and concentrates, and were milked twice daily. Animals were blocked by parity and DIM and randomly assigned to 2 groups ($n = 17$ /group): control (CTR) and spice mix-supplemented group (SFA). Cows in SFA group were supplemented with 2 g/d of a spice feed additive (Oleobiotec Saliva Promoter, Laboratoires Phodé, France), mixed with the TMR, for 28 d. Feed intake was measured daily, milk yield was recorded at each milking, and milk samples were collected weekly to determine milk composition and SCC. A subset of cows ($n = 10$ /group) was fitted with a reticulorumen sensor equipped with a pH reader. Data were analyzed with repeated measures mixed models using the GLIMMIX procedure (SAS 9.4). DMI was overall unaffected by treatment (28.0 vs. 27.4 ± 0.57 kg/d for CTR and SFA, respectively; $P = 0.45$). Feeding behavior did not significantly differ between groups. Milk yield, ECM yield, milk composition, and SCC did not differ between groups. Feed efficiency was unaltered as well (1.57 vs. 1.58 ± 0.04 kg milk/kg DMI, $P = 0.88$). Rumen pH was numerically higher in SFA compared with CTR (6.08 vs. 6.19 ± 0.07 in CTR and SFA, respectively; $P = 0.29$), with a significant interaction Trt $\times$ Time effect ($P < 0.01$). The pH pattern throughout the day was different between groups, with greater values in SFA ($P < 0.01$). In these conditions (mid lactating cows in thermoneutrality), the supplementation of a spice feed additive did not impact performance but slightly increased rumen pH.

Key Words: spice extract, behavior, rumen pH

2724 Energy partitioning and efficiency of dairy cows in 3 different feeding systems. A. Jasinsky*, A. Casal, and M. Carriquiry, *Facultad de Agronomía-Universidad de la República, Montevideo, Uruguay*.

To compare the effect of 3 feeding systems (FS) on energy partitioning between heat production (HP) and retained energy (RE) in milk and body tissue during the first 120 d of lactation, 36 multiparous fall-calved Holstein cows were used. Cows were blocked according to means $\pm$ SD calving date ($03/22/2019 \pm 14$), BW (670 ± 77), and BCS (3 ± 0.46) and randomly assigned in an incomplete factorial design to 1 of 3 FS:

cows in a compost barn (CB-TMR) fed TMR ad libitum, grazing cows supplemented with TMR in a compost barn (CB-GRZ), or in an open-sky pen (OP-GRZ). Cows were milked twice a day, and milk yield was recorded daily; weekly samples were collected for milk composition analyses. Cow BW was recorded monthly and BCS every 2 wk. Heat production was measured using the heart rate- O_2 pulse technique at 30 and 90 ± 14 d. Data were analyzed with a mixed model with FS as fixed effect, means were considered to differ when $P < 0.05$. Milk yield was greater for CB-TMR than GRZ cows (42.9 vs. 34.5 ± 1.1 kg/d, respectively), but milk composition did not differ, thus, RE-milk was greater for CB-TMR than GRZ cows. No differences between FS were observed in BW ($P = 0.36$), but average BCS was greater for CB-TMR than GRZ cows (2.8 vs. 2.6 ± 0.04 units, respectively; $P = 0.04$), however, RE-tissue did not differ between FS ($P = 0.73$). Total RE, HP, and ME intake (total RE + HP), expressed as unit of $BW^{0.75}$ were greater for CB-TMR than GRZ cows (995 vs. 805 ± 28 , $1,049$ vs. 977 ± 22 , and $2,043$ vs. $1,780 \pm 46$ kJ/kg $BW^{0.75}$ /d, respectively; $P < 0.05$). Therefore, adjusted gross energy efficiency (total-RE/ME intake) was greater for CB-TMR vs. GRZ cows (0.49 vs. 0.45 ± 0.006 , respectively; $P < 0.01$). Cows fed 100% TMR in a CB produced more milk, maintained a greater BCS, and partitioned more of the ME consumed into milk. However, no effect of environmental exposure during supplementation of GRZ cows (CB vs. OP) were observed in energy partitioning and efficiency.

Key Words: energy expenditure, grazing, environmental exposure

2725 Evaluating the responsiveness to propionic acid of grass hay baled at 80% DM across a gradient of alfalfa inclusion. B. Escudero Alejos*, J. B. Poblete¹, M. Chusho Guevara¹, A. P. Jimenez Lagos¹, J. M. Sandro¹, K. Nishimwe¹, D. Zamudio Ayala¹, M. V. Cardoso¹, C. Knight², S. L. Annis³, J. Garzon², G. Pereira², H. R. L. Neto⁴, E. Rodberg⁴, J. J. Romero¹, ¹*Animal and Veterinary Sciences, School of Food and Agriculture, University of Maine, Orono, ME*, ²*University of Maine Cooperative Extension, Orono, ME*, ³*School of Biology and Ecology, University of Maine, Orono, ME*, ⁴*Kemin Industries, Des Moines, IA*.

Our objective was to determine the effects of the forage mixture (ALF), dose of propionic acid (DOSE), and storage stage (STG) on hay preservation. Forage mixtures had 4 levels: 0/100%, 35/65%, 65/35%, and 100/0% of alfalfa/timothy grass. Propionic acid was applied at 0%, 0.25%, and 0.5% (fresh basis), and STG were d 0 (start) and 40 (end). Data were analyzed as a randomized complete block design (5 field blocks) with a 4 ALF $\times$ 3 DOSE $\times$ 2 STG factorial arrangement using the PROC GLIMMIX of SAS v.9.4. For heating measures, day was a repeated measure. Mini bales were prepared in this study (bale weight: $\bar{x}$ -bar = 498.4 ± 5.77 g fresh basis; density: $\bar{x}$ -bar = 275.3 ± 5.9 kg DM/m³). Differences were declared at $P \leq 0.05$. The DM increased from d 0 to 40 (79.9 to $82.0 \pm 0.21\%$; STG). After 40-d storage, DM losses were higher for 0% DOSE ($4.23\% \pm 0.454\%$) vs. 0.25% and 0.5% ($\bar{x}$ -bar = 1.76). For both STG, at 0.5% DOSE, ALF 65% had higher mold counts than 0% and 100% (5.84 vs. $\bar{x}$ -bar = 5.07 ± 0.204 log cfu/g fresh, respectively) but was no different than ALF 35% (5.34); DOSE 0% ($\bar{x}$ -bar = $6.53 \pm 0.204^\circ\text{C}$) and 0.25% ($\bar{x}$ -bar = $5.86 \pm 0.204^\circ\text{C}$) were not affected by ALF (ALF $\times$ DOSE). For all ALF at d 40, as DOSE increased, yeast count decreased (5.15 , 4.55 , and 4.05 ± 0.136 log cfu/g fresh, respectively; DOSE $\times$ STG). For all DOSE at d 40, ALF 100% had the lowest yeast count compared with all other ALF levels (4.0 vs. $\bar{x}$ -bar = 4.78 ± 0.151 log cfu/g fresh, respectively; ALF $\times$ STG). ALF or DOSE did not affect maximum bale temperature across the 40 d ($\bar{x}$ -bar = $24.2 \pm 0.36^\circ\text{C}$). Across all ALF, DOSE 0.5% had a lower bale temperature than 0% from d 15 to 40 ($\Delta = 1.38 \pm 0.192^\circ\text{C}$), and 0.5%

had a lower bale temperature than 0.25% from d 25 to d 40 ($\Delta = 0.479 \pm 0.192^\circ\text{C}$). In conclusion, applying 0.5% propionic acid reduced DM losses, yeast counts, and bale temperature in grass hay stored at 80% DM, regardless of the alfalfa inclusion rate. Lower bale temperatures suggest improved preservation and reduced microbial spoilage risk. These results support the use of propionic acid-based preservatives to enhance hay stability when baling DM is around 80%.

Key Words: alfalfa hay, grass hay, preservative

2726 Tumor necrosis factor- α 's role during the periparturient period. R. Hernandez*, C. Haines, Z. Hong, D. B. Vagnoni, and K. E. Vagnoni, *California Polytechnic State University, San Luis Obispo, CA*.

Because there is increasing interest in the role of periparturient inflammation in transition cow metabolic disorders (e.g., hypocalcemia, ketosis), we evaluated the relationships among serum concentrations of tumor necrosis factor- α (TNF α), haptoglobin, and calcium in cows through the transition period. Sixty-three cows (32 Jersey and 31 Holstein) of first (16), second (16), or third (15), and fourth or greater (15) parity were enrolled in a longitudinal study with frequent blood samples obtained from 28 d before expected parturition to 7 d postpartum. The relationships among these metabolites were evaluated using mixed linear models including the effects of breed, parity group (1, 2, 3, or ≥ 4), day, and their interactions, with day as a repeated factor. Additionally, TNF α values concurrent, 1 d preceding, and 1 d following calcium and haptoglobin concentrations were evaluated as covariates to determine the temporal relationships among these variables. Haptoglobin and TNF α were log-transformed before statistical analyses to stabilize the variances. Serum TNF α concentrations were higher ($P = 0.03$) for Jerseys than for Holsteins and also differed due to day ($P < 0.001$), generally decreasing during the prepartum period and then remaining relatively stable thereafter. Serum calcium concentrations were affected ($P < 0.001$) by both day and the interaction of parity group with day; concentrations reached their nadir on d 1 postpartum, and nadir values successively decreased with increasing parity group. Serum haptoglobin concentrations were affected ($P \leq 0.002$) by both day and the interaction of breed with day, increasing rapidly postpartum and more so for Jerseys than Holsteins. When serum TNF α was added as a covariate to the models for serum calcium and haptoglobin, model fit (as assessed by both Akaike and Bayesian information criteria, as well as the log-likelihood function) improved and statistical significance of the covariate term increased as TNF α values shifted from the day following to concurrent, to the day preceding. Results indicated that TNF α changes preceded changes in haptoglobin and calcium as cows move through the transition period.

2727 Effect of a blend of ginger, lemon balm, cinnamaldehyde, inactivated yeasts, and clays on the rumen microbiome. S. M. Tondini*¹, M. Romero-Huelva², E. Ramos-Morales², P. Romero², E. Jiménez², H. Khelil-Arfa¹, R. Breitsma¹, A. Blanchard¹, and D. R. Yáñez-Ruiz², ¹ADM International Sàrl, Rolle, Switzerland, ²Estación Experimental del Zaidín, CSIC, Granada, Spain.

The objective was to evaluate the effects of a blend consisting of ginger, lemon balm, cinnamaldehyde, inactivated yeasts, and clays (AF) on the rumen microbiome using an in vitro continuous culture system. The trial consisted of 2 replicated 18-d incubation periods beginning with 5 d of adaptation before the administration of 1 of 2 treatments: (1) CON; no supplementation and (2) AF supplemented at 0.125 g/d. The basal diet consisted of 60% forage (corn silage + grass silage) and 40% concentrate (barley, corn, sunflower, urea). On d 16, samples were collected from

each vessel ($n = 8$) and snap frozen in liquid nitrogen. Total microbial DNA was extracted and sequenced using universal primers for amplification of the 16S rRNA gene. Taxonomic assignment of amplicon sequence variants (ASVs) was performed against the Greengenes2 database. Relative abundance of bacteria present at 0.3% or above was analyzed using a mixed model with fixed effects of treatment, period, and their interaction. Vessel was included as a random effect. Measures of richness and diversity, including Chao1, Simpson, and Shannon indices were not affected ($P \geq 0.53$) by treatment, but AF numerically increased each metric. At the phylum level, 84% of all ASVs were assigned to *Bacteroidetes* (33%), *Firmicutes* (29%), or *Proteobacteria* (26%). There was a tendency ($P = 0.06$) for *Verrucomicrobia* to have greater relative abundance due to AF supplementation. At the genus level, AF increased ($P < 0.01$) the relative abundance of *Prevotella* by 2.4-fold compared with CON. Members of the genus, *Prevotella*, are the most numerically dominant in the rumen and possess a wide array of functional capabilities. At the species level, AF increased the relative abundance of *Prevotella communis* and decreased the relative abundance of *Prevotella ruminicola* when compared with CON. Overall, supplementation with AF does not impact microbial richness and diversity but may alter the profile of rumen bacterial communities.

Key Words: rumen microbiome, bioactive, yeast

2728 Fermentation Explorer: An online app for analyzing microbiome datasets from the rumen and other environments. T. Hackmann*, *University of California, Davis, Davis, CA*.

Microbes in the rumen of cattle ferment feed, which releases short-chain fatty acids to the host and methane as a byproduct. Microbiome sequencing suggests many organisms are involved in this process, but their specific roles are unclear. To overcome this bottleneck, we have developed Fermentation Explorer, an app for predicting roles of fermentative microbes in microbiome datasets. Our app was first made available in 2023, and here we describe the latest version. The app has 3 tools that predict a total of 25 roles (traits). The tools rely on name matching, building metabolic networks, and machine learning. The traits include those related to metabolism (e.g., types of end products), physiology (e.g., oxygen tolerance), morphology, and habitat. We demonstrate use of the app with a dataset from continuous culture fermentors. These fermentors were inoculated with rumen microbes, fed glucose or cellulose, and subjected to 16S rDNA sequencing. The app predicted key differences between microbes in these fermentors, including substrates for fermentation. We expect this app to be a useful resource for the community in analyzing their own datasets. The app is available as a web server and for download at <https://github.com/thackmann/FermentationExplorer>.

Key Words: rumen, fermentation, microbiome

2729 Purple-corn silage: II. Effects of feeding anthocyanins to multiparous Holstein dairy cows on lactation performance and somatic cell count. O. I. Santana*¹, F. J. Santoyo Ávila², J. I. Sánchez Duarte³, and A. Peña Ramos¹, ¹Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Pabellón de Arteaga, Aguascalientes, Mexico, ²Universidad Autónoma de Aguascalientes, Jesús María, Aguascalientes, Mexico, ³Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Matamoros, Coahuila, Mexico.

The aim of this study was to evaluate the effect of feeding purple-corn silage on lactation performance and SCC of multiparous dairy cows. Four pens of 32 Holstein cows each were balanced by 2.3 lactations, 79

± 11 DIM, and 637 ± 21 kg BW. A 2-replicated randomized complete design was used to feed white-dent kernel silage (WS) or purple silage (PS) from an isogenic hybrid (Supremo, Aspros). Forage was fermented for 64 d, and the PS had a mean concentration through the whole trial of 796 ± 120 mg/kg of anthocyanins analyzed as cyanidin-3-glucoside. All cows were fed with a control diet using WS in a 2-wk covariate period followed by 8 experimental weeks in which 2 pens were assigned to either WS or PS treatment. Diets contained (DM basis) 27% WS or PS, 17% alfalfa hay, 30% ground corn grain, 24% concentrate, and 2% of mineral premix. Diets were iso-nitrogenous (16.7% CP), iso-energetic (1.61 Mcal/kg), iso-fibrous (30.1%), and iso-starch (37%). Milk samples were taken from 8 cows per treatment on d 11 and 12 of covariate period, and on d 4 and 5 of wk 2, 4, 6, and 8. Milk samples were analyzed for milk composition and SCC. Data were analyzed in R-Studio in a model that included covariate period, fixed effects of diet and week, random effect of pen, and the interaction diet $\times$ week. Cows fed PS consumed less DM (28.6 kg/d) than cows fed WS (29.5 kg/d). No differences were detected on milk yield, but through 8 experimental weeks, cows fed PS yielded persistently between 0.3 and 0.6 kg more milk than cows fed WS (41.8 kg/d vs. 41.4 kg/d, respectively). Hence, feed efficiency was greater for cows fed PS than for WS (1.46 vs. 1.40, respectively). Milk composition did not differ between treatments and values were within the normal range. Level of SCC was affected by the interaction diet $\times$ week, as cows fed PS kept similar SCC through study ($53 \pm 18 \times 10^3$ cell/mL), but cows fed WS were from 2-fold to 3-fold greater SCC in wk 2 and 4 compared to wk 6 and 8, respectively. In conclusion, feeding PS significantly decreased SCC, whereas DMI descended without affecting milk yield.

Key Words: anthocyanin, dairy cow, silage

2730 Fecal n-alkane in Holsteins fed a high or low hay ration. W. Yousaf*, Y. Adeniji, K. J. Harvatine, W. B. Staniar, and C. D. Dechow, *Department of Animal Science, Pennsylvania State University, University Park, PA.*

Selection for feed efficiency could be biased if there is feed sorting. This study was designed to evaluate if fecal n-alkanes could be used to assess variation in the diet ingredients a cow consumes by altering the level of hay provided in a TMR. Five primiparous and 5 multiparous cows (>100 DIM) were grouped randomly in a crossover design with a 10-d adaptation period followed by 2 d of sample collection. Two dietary treatments were fed ad libitum with a target of 5% refusals: low hay (LH = 98% base TMR + 2% hay) and high hay (HH = 90% base TMR + 10% hay). Fecal samples were collected in the morning and evening before milking on each sampling day. Individual diet ingredients, TMR, and individual fecal samples were evaluated for n-alkane concentrations (mg/kg DM). The primary n-alkanes considered were n-nonacosane (C29), n-hentriacontane (C31), and n-tritriacontane (C33) because of relatively high concentration in forages. Data were analyzed using a mixed model with treatment as a fixed effect and cow as a random effect. Mean concentration of C29, C31, and C33 was higher in HH

TMR (34.36, 64.29, and 16.29 mg/kg DM, respectively) when compared with LH TMR (31.59, 57.87, and 13.79 mg/kg DM, respectively). Fecal concentrations of C29, C31, and C33 were higher for HH ($P < 0.05$; 70.56 ± 3.17 , 150.91 ± 6.65 , and 37.58 ± 1.48 mg/kg DM, respectively) than for LH (65.13 ± 3.17 , 135.12 ± 6.65 , and 32.20 ± 1.48 mg/kg DM, respectively). We concluded that naturally occurring fecal odd chain n-alkanes reflected the diet cows consumed and may be a tool to assess feed sorting differences among cows.

Key Words: fecal alkane, feed sorting

2731 Correlations of rumen pH with performance and health of lactating cows. J. P. A. Rezende*, L. C. B. Pimenta, M. C. S. Santos, G. M. de Souza, and M. A. C. Danes, *Universidade Federal de Lavras, Lavras, MG, Brazil.*

We aimed to explore the correlations of rumen pH recorded by boluses (smaXtec) with production and health parameters of dairy cows. Data from 21 Holstein cows (DIM = 144 ± 77) monitored for 9 wk were used, with data collection every 3 wk, totaling 63 observations. Rumen pH (recorded every 10 min) averages from wk 3, 6, and 9 were used to calculate mean pH, time below pH 6, 5.8, and 5.5, time below pH 6/DMI, 5.8/DMI, maximum pH, minimum pH, and Δ pH (maximum – minimum pH), that were compared with milk yield (MY), ECM, DMI, milk fat (FY) and protein yield (PY), milk fatty acids (FA), serum haptoglobin (Hp), and fecal pH. Pearson correlations were calculated using SAS, with significance set at $r \geq 0.3$ or ≤ -0.3 and $P < 0.05$. All reported correlations meet these significance criteria. On average $\pm$ SD, MY was 34.7 kg/d ± 5.3 and mean pH was 6.02 while ± 0.17 . Time below pH 6, 5.8, and 5.5 (min/d) was 634 ± 396 , 292 ± 279 , and 49 ± 88 , respectively, time below pH 6/DMI and 5.8/DMI was 30 ± 20 and 14 ± 14 . Maximum, minimum, and Δ pH were 6.41 ± 0.18 , 5.58 ± 0.21 , and 0.83 ± 0.23 . Mean pH correlated positively with MY ($r = 0.54$), ECM (0.57), FY (0.52), and PY (0.44), and negatively with C18:1 *cis*-9 (-0.32). Time below pH 6 correlated negatively with MY (-0.54), ECM (-0.56), FY (-0.50), and PY (-0.43), and positively with C13:0 anteiso (0.30) and C18:1 *cis*-9 (0.33). Time below pH 5.8 correlated negatively with MY (-0.38), ECM (-0.45), FY (-0.43), and PY (-0.36), and positively with C18:1 *cis*-9 (0.37). Time below pH 5.5 correlated with Hp (0.38). Time below pH 6/DMI correlated with MY (-0.63), ECM (-0.67), DMI (-0.48), FY (-0.58), PY (-0.59), and de novo FA (-0.30). Time below pH 5.8/DMI correlated negatively with MY (-0.45), ECM (-0.54), DMI (-0.38), FY (-0.49), PY (-0.48), and positively with C18:1 *cis*-9 (0.32). Maximum pH correlated with MY (0.54), ECM (0.55), FY (0.47), PY (0.41), and negatively with C18:1 *cis*-9 (-0.38). Minimum pH correlated with MY (0.34), ECM (0.36), and FY (0.39). The Δ pH correlated with fecal pH (-0.31). The strongest performance correlations were with time below pH 6/DMI. Only time below pH 5.5 correlated with Hp, whereas Δ pH was the only one related to fecal pH.

Key Words: acidosis, bolus

Ruminant Nutrition 3: Methane—Posters

2732 Evaluation of novel methane inhibitors in vitro. A. Richards*, C. Eifert, N. Stepanchenko, C. Vidal de Almeida, S. Issabekova, Y. Kim, and A. N. Hristov, *The Pennsylvania State University, State College, PA*.

Enteric CH₄ production in the rumen represents both an environmental concern and an energy loss to the host animal. Therefore, identifying and evaluating novel inhibitors is essential to mitigate CH₄ emissions more effectively. The objective of this study was to evaluate the effects of several novel inhibitors on enteric CH₄ production in vitro. Twelve, 24-h batch-culture incubations using an automated gas production system were conducted with rumen fluid collected from 2 ruminally cannulated lactating Holstein cows. Total mixed ration (70% forage and 30% concentrate feeds) fed to donor cows was also used as fermentation substrate, included at 1% (wt/vol) in the incubation medium. Incubations were carried out for 24 h at 39°C with continuous agitation, and each incubation included a negative control (NEG; TMR without additives), and a positive control (POS; TMR with 2.0 mM chloroform). Gas samples were collected at 12 and 24 h for analysis of CH₄ and H₂ concentrations. The PROC MIXED procedure of SAS with fixed effect of treatment was used to analyze the data. Out of the 83 inhibitors tested, 22 were found to decrease ($P < 0.001$) CH₄ production (as % of total gas) by up to 98% at inclusion rate at or below 500 mg/kg TMR. Fifteen of the inhibitors that decreased CH₄ production also decreased ($P < 0.001$) total gas production (TGP) by 14% to 31%. Several compounds had no effect ($P \geq 0.10$) on TGP but decreased CH₄ emission by 25% to 95%. The efficacy of 6 of these compounds increased ($P < 0.001$) as incubation progressed, although TGP remained similar ($P \geq 0.07$) to NEG. Three of the 6 compounds, AR8, AR12, and AR26, reduced CH₄ production 37% to 98% of that for NEG at 100 mg/kg TMR DM inclusion rate (Table 1) and were considered viable for investigating further in animal studies.

Key Words: methane, inhibitor, in vitro

2733 Characterizing the number of spot samples required for accurate determination of gaseous fluxes and heat production in dairy cows using the GreenFeed system. M. A. Rahman*, W. M. Sims, M. M. H. Khandakar, M. G. M. Carvalho, R. A. Menzes, M. A. Arshad, O. Osulale, and A. F. Brito, *Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, NH*.

Previous research showed that the GreenFeed (GF) system can accurately measure gaseous emissions in experiments done with ruminants. However, determining the number of gaseous spot samples (SS) needed to improve measurement accuracy could further strengthen the use of this technology. We aimed to determine the number of SS required per cow to accurately estimate gaseous fluxes (CH₄, CO₂, O₂, and H₂) and

heat production (HP) in lactating dairy cows. In trial 1, 10 multiparous and 6 primiparous (51 ± 26 DIM) organic-certified Jersey cows were used and fed a TMR consisting of (DM basis) 60% grass-legume baleage and 40% concentrate mix. In trial 2, the same cows (104 ± 27 DIM) were used and fed a diet consisting of (DM basis) 60% grass-legume baleage, 37.5% concentrate mix, and 2.5% flaxseed oil. Diets averaged (DM basis) 15.8% CP, 32% NDF, and 4.04% total FA in trial 1, and 16% CP, 34% NDF, and 5.68% total FA in trial 2. Diets were fed for 13 wk (7 wk in trial 1 and 6 wk in trial 2). One GF unit was used to quantify 100 SS from each cow during both trials. Spot urinary samples were taken 5 times over 3 d each week to measure N concentration for calculating HP. The Pearson and Spearman correlations were performed between variable responses (gaseous fluxes and HP) and the first 10 visits, and increments of 10 visits up to 100 (forward = FD). Similar calculations were made in a reversal order from 100 to 10 visits (reverse = RV). The minimum number of SS was determined based on FD and RV visits when the correlation values were 0.95 or greater. In trial 1, the minimum number of SS required for accurate quantification of CH₄, CO₂, O₂, and H₂ were 50, 30, 70, and 50, respectively, obtained in 13.3 ± 3.42 d. Fifty SS were required to accurately estimate HP, which was achieved in 14 ± 1.96 d. In trial 2, the minimum number of SS required for accurate quantification of CH₄, CO₂, O₂, and H₂ were 50, 50, 40, and 90, respectively, accomplished in 15.4 ± 2.28 d. Thirty SS were needed for accurate estimation of HP, achieved in 8 ± 1.13 d. In conclusion, the number of SS needed for accurate measurements varied by individual gas and type of diets.

Key Words: GreenFeed, heat production, methane

2734 Feeding *Chondrus crispus*: Effects on production performance and nutrient digestibility in grazing dairy cows. M. A. Rahman*, W. M. Sims, M. L. Fischer, M. A. Arshad, M. M. H. Khandakar, M. G. M. Carvalho, L. C. R. Junior, O. Osulale, and A. F. Brito, *Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, NH*.

We aimed to investigate the effects of the red seaweed *Chondrus crispus* on production performance and nutrient digestibility in dairy cows. Twelve multiparous and 8 primiparous organic-certified Jersey cows (136 ± 32 DIM, 442 ± 52 BW) were used in a randomized complete block design. Cows had access to a grass-legume pasture mix (herbage allowance = 17.5 kg/cow daily) for 16 h/d and housed for 8 h/d when they received a partial TMR (pTMR) and were milked. The pTMR was formulated to contain (DM basis): 37% mixed-mostly legume baleage and 63% of a ground-corn/soybean meal-based concentrate. Cows were paired ($n = 10$ pairs) based on DIM and parity and, within block, randomly assigned to 1 of 2 diets: (1) pasture plus pTMR (control = CON) or (2) pasture, pTMR, and 3% *C. crispus* (seaweed = SWD). Diets

Table 1 (Abstr. 2732). Effect of novel CH₄ inhibitors (applied at 100 mg/kg TMR DM) on total gas and CH₄ production in vitro

Item	Treatment ¹					SEM	P-value
	POS	NEG	AR8	AR12	AR26		
Total gas, mL/g of substrate DM	149	142	126	140	120	6.68	0.06
CH ₄ ^a , % of total gas	0.099 ^c	4.95 ^a	3.61 ^b	3.37 ^b	0.115 ^c	0.240	<0.001

^{a-c}Within row, means with different superscripts differ ($P \leq 0.05$).

¹Treatments were run in triplicates ($n = 3$).

were formulated to be isonitrogenous and comparable mineral profile with a 60:40 forage:concentrate ratio. Pasture averaged 16.3% CP and 55.2% NDFom, with the pTMR averaging 16% CP and 25% NDFom. The experiment lasted 10 wk, with 2 wk for covariate measurements followed by 2 sampling periods during wk 4 and 8. Herbage DMI was estimated using Cr₂O₃ and in vitro DM digestibility of feeds. Fecal grab samples were taken 10 times over 6 d in each sampling period, with Cr₂O₃ used for estimating fecal output of DM and apparent total-tract digestibility of nutrients. Herbage and pTMR intake were greater ($P \leq 0.03$) in SWD than CON. Consequently, the intake of DM, OM, CP, and aNDFom all increased ($P \leq 0.04$) with feeding SWD versus CON. Diets did not affect ($P \geq 0.35$) milk yield and the concentrations of milk fat and MUN. However, compared with CON, SWD cows had decreased ($P < 0.05$) concentrations of milk true protein, lactose, and SNF, and yields of milk fat, true protein, lactose, 4% FCM, and ECM. Except for the digestibility of OM, which decreased ($P = 0.01$) in cows fed SWD versus CON, diets did not affect ($P \geq 0.17$) digestibilities of DM, CP, aNDFom, and ADF. In summary, *C. crispus* fed at 3% of the diet DM negatively affected the concentrations and yields of most milk components despite increased DMI.

Key Words: digestibility, milk, seaweed

2735 Evaluating taste preference of *Asparagopsis taxiformis* in dairy cattle. M. A. Rahman*, M. L. Fischer, M. A. Arshad, W. M. Sims, O. Osulale, and A. F. Brito, *Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, NH.*

We evaluated the taste preference of the red seaweed *Asparagopsis taxiformis* (AT) with different processing methods using dairy cattle in a sequential elimination experiment. Four pregnant Holstein heifers (624 ± 61 kg BW) and 2 multiparous Holstein dry cows (644 ± 85 kg BW) were used in the study. Treatments consisted of TMR (73:27 forage:concentrate ratio; 15% CP, 38% NDF) without AT (control = CON) or TMR supplemented with 150 g/d AT emulsified in sunflower oil (AT-SUN), 139 g/d AT emulsified in canola oil (AT-CAN), or 28 g/d freeze-dried AT (FD-AT). Treatments containing AT were formulated to deliver 325 mg of bromoform daily. One animal was enrolled per time in the study, with test lasting 11 d. The feeding regimen consisted of d 0–2 (adaptation phase), d 3–6 (feeding segment 1), d 7–9 (feeding segment 2), and d 10–11 (feeding segment 3). Each animal was fed once daily, with all treatments offered simultaneously for the first 6 d. After segment 1, the most preferred treatment was removed, with the remaining 3 treatments offered in segment 2. This process was repeated until the final 2 d of the study when only the 2 least preferred treatments were offered. Data tabulation and calculations were done in Microsoft Excel. Actual bromoform supply averaged 413 mg/d in AT-SUN and AT-CAN, and 241 mg/d in FD-AT. During segment 1, CON was the most consumed diet resulting in a total DMI of 120.4 kg, followed by AT-SUN, FD-AT, and AT-CAN averaging 90.3, 66.0, and 59.3 kg total DMI, respectively. Similarly, CON was the most preferred treatment during segment 1 totaling 11 d as the animals' first dietary choice, with AT-SUN, AT-CAN, and FD-AT totaling 6, 4, and 3 d as the top selected treatment, respectively. When all 3 feeding segments were evaluated for generating the final ranking, CON was the top choice of 4 animals averaging 1.33 points on a scale ranging from 1 (most preferred) to 4 (least preferred), followed by AT-SUN (2.17 points), AT-CAN (3.0 points), and FD-AT (3.50 points), respectively. Overall, our results showed that pregnant heifers and dry cows were able to differentiate among various processed forms of AT based on taste preference.

Key Words: dairy cattle, preference, seaweed

Table 1 (Abstr. 2736). Effect of Brominata pellets on milk yield and components, intake, and digestibility of lactating Holstein cows

Item	Control	Treatment	SEM	P-value
Milk yield, kg/d	23.03	24.21	0.586	0.18
Feed efficiency, kg/kg	1.00	1.06	0.054	0.43
Fat, %	4.62	4.70	0.427	0.89
Protein, %	3.28	3.35	0.137	0.73
Lactose, %	4.46	4.50	0.116	0.82
Solids nonfat, %	7.74	7.84	0.301	0.71
DMI, kg/d	21.93	22.02	1.078	0.94
OMI, kg/d	20.05	20.12	2.337	0.94
Fat intake, kg/d	1.29	1.26	0.064	0.62
DM digestibility, g/g	0.72	0.70	0.0068	0.13
OM digestibility, g/g	0.721	0.708	0.0065	0.17
Fat digestibility, g/g	0.90	0.87	0.0119	0.04

2736 Effects of a pelletized mash containing fresh *Asparagopsis taxiformis* "brominata" on the dry matter intake of dairy cows. I. F. Carrari*, V. A. Achziger¹, N. V. Tropia⁴, D. R. Andrade⁴, and M. I. Marcondes^{1,2}, ¹Washington State University, Pullman, WA, ²William H. Miner Institute, Chazy, NY, ³Blue Ocean Barns, San Diego, CA, ⁴Universidade Federal de Viçosa, Viçosa, MG, Brazil.

Enteric methane emissions from ruminants are a primary environmental concern, and the seaweed *Asparagopsis taxiformis* has been proposed as a mitigation strategy. However, its effects on DMI and animal performance require further investigation. This study evaluated the impact of Brominata pellets, a bromoform-stable fresh *A. taxiformis* product, on the DMI, digestibility, selectivity, milk production, and feed efficiency of lactating Holstein cows. Twelve lactating cows (254 ± 54.2 DIM) housed individually were assigned to 1 of 2 treatments: control or Brominata. The study lasted 23 d, including a 7-d adaptation period, an 8-d ramp-up phase, and 8 d of full-dose feeding (200 g/d). Milk samples (of final 3 d) were analyzed for composition, and digestibility was evaluated using spot fecal collection. Data were analyzed using a randomized block design. Results (Table 1) indicated no significant differences in DMI, milk yield, or feed efficiency between treatments ($P > 0.05$). Also, digestibility analyses revealed a significant reduction in fat digestibility ($P = 0.046$) in cows fed Brominata. Sorting analysis did not show selective avoidance or preference for Brominata pellets ($P > 0.05$). We concluded that the inclusion of Brominata pellets did not negatively impact DMI, milk yield, or feeding behavior, suggesting that it is a promising methane-reducing feed additive. Overall, these results support the potential for Brominata to improve the environmental sustainability of dairy production without compromising performance.

Key Words: digestibility, performance, seaweed

2737 Effects of combining nitrate and tannins for methane mitigation in dairy cows. R. Peng*, S. E. Räisänen², R. Rauch³, A. Rytz³, E. Soussan³, M. Bobkov³, M. Kreuzer¹, and M. Niu¹, ¹Department of Environmental Systems Science, Institute of Agricultural Sciences, ETH Zürich, Zürich, Switzerland, ²Department of Animal and Veterinary Sciences, Aarhus University, Tjele, Denmark, ³Nestlé Institute of Agricultural Sciences, Nestlé Research, Lausanne, Switzerland.

This study evaluates the combined effects of nitrate and *Acacia mearnsii* tannins (AMT) on nutrient digestibility, milk production, and enteric methane (CH₄) emission. It also examines if the negative effects of AMT could be offset by using a rumen-protected protein source (RPR;

rapeseed meal). Eighteen Holsteins (203 ± 60.5 d in milk, 33 ± 5.0 kg/d milk yield) fed a low-CP diet (14% of DM) were used in a split-plot design with nitrate (0.62% DM) or an isonitrogenous urea inclusion as the main plot treatments. Cows transitioned immediately from the pre-experimental diet to the final dietary nitrate content without any step-up. Within each plot, cows followed a replicated 3×3 Latin square with 3 treatments: (1) CON (basal diet; 21% starch, 38% NDF), (2) AMT (3% of DM; 21% starch, 37% NDF), and (3) AMT+RPR (6.4% of DM; 16% starch, 39% NDF). Eight spot fecal samples and gas emissions (GreenFeed system) were collected at the end of each 21-d period. Data were analyzed using a linear mixed model in R. Significant ($P < 0.01$) interactions between main plot and subplot treatments were observed for fiber intake and digestibility of DM, OM, and NDF, but not for energy-corrected milk (ECM) yield. In the urea plot, AMT reduced ($P < 0.01$) digestibility of OM, CP and NDF by 17%, 30%, and 39%, respectively, but the magnitudes of reductions were approximately 2-fold smaller in the nitrate plot. Extra RPR did not offset the adverse effects of AMT on digestibility, milk yield, or composition. Nitrate increased ($P < 0.01$) milk yield (28 ± 0.5 vs. 25 ± 0.5 kg/d) over urea but did not affect ECM yield or milk composition. Nitrate reduced ($P = 0.01$) CH_4 intensity (g/kg ECM yield) by 12% to 13% but did not affect CH_4 production or CH_4 yield. Across main plots, AMT reduced ($P < 0.01$) ECM yield from 29 ± 1.0 to 27 ± 1.0 kg/d compared with CON. Extra RPR with AMT failed to mitigate these adverse effects, possibly due to lower starch content in AMT+RPR diets, limiting energy availability and exacerbating digestibility challenges. Overall, nitrate reduced CH_4 intensity but not CH_4 yield or daily production. Supplementing AMT did not lower methane intensity, yield, or daily production but decreased ECM yield, and additional RPR did not offset these negative effects of AMT.

Key Words: cattle, enteric methane

2738 Production performance, fiber digestion, and methane emissions of cows consuming triticale silages of different maturities. H. Galyon* and G. Ferreira, *Virginia Tech, Blacksburg, VA.*

This study aimed to analyze production performance, fiber digestibility, and methane emissions of lactating Holstein cows consuming diets containing triticale silage harvested at either boot stage (BS) or soft-dough stage (SDS) of maturity. Cows (16 primiparous, 8 multiparous) were assigned to 1 of 2 diets (BS vs. SDS) in a crossover design over 28-d periods. The diets were formulated such that triticale silage, corn silage, and concentrate were each a third of the dietary NDF (33% DM) and contained 19% triticale silage, 25% corn silage, and 56% concentrate. Digestibilities of DM (DMD), NDF (NDFD), and potentially degradable NDF (pdNDFD) were determined using undegradable NDF (uNDF) as an internal marker. Half of the cows were rumen-cannulated to assess the VFA profile in the ruminal fluid. Methane production (CH_4P), yield (CH_4Y), and intensity (CH_4I) of noncannulated cows were calculated via the GreenFeed system. Using the PROC MIXED of SAS, the model included the fixed effects of silage and the random effects of cow and period. We observed no difference in DMI (24.9 kg/d) or NDF intake (9.0 kg/d) between silages. However, cows consuming BS exhibited a 0.6-kg/d reduction in uNDF intake ($P < 0.01$) and a 0.6-kg/d increase in pdNDF intake ($P < 0.01$) relative to cows consuming SDS. Cows consuming BS had greater DMD (64.1% vs. 57.8%; $P < 0.01$), NDFD (51.8% vs. 43.7%; $P < 0.01$), and pdNDFD (65.4% vs. 60.4%; $P < 0.01$) and digested more DM (16.1 vs. 14.2 kg/d, $P < 0.01$) and NDF (4.7 vs. 3.9 kg/d, $P < 0.01$) than cows consuming SDS. Cows consuming BS produced 3.4 kg/d more ECM ($P < 0.01$) than cows consuming SDS, although milk fat (4.5%) and milk protein (3.2%) did not differ. Cows

consuming BS had greater total VFA (166 vs. 151 mM, $P < 0.01$) and a lower acetate-to-propionate ratio (3.8 vs. 4.0, $P = 0.03$) than cows consuming SDS. Cows consuming BS had less CH_4P (332 vs. 348 g/d; $P = 0.03$) and CH_4I (7.8 vs. 8.7 g/kg ECM) than cows consuming SDS, but CH_4Y was not different (14.1 g/kg DMI, $P = 0.08$). In conclusion, feeding BS triticale silage, relative to SDS silage, enhanced fiber digestion and ruminal VFA concentrations and reduced CH_4 production.

Key Words: triticale, forage maturity, methane

2739 Effects of 2-bromoethanesulfonate alone or in combination with monensin or calcium propionate on in vitro CH_4 and H_2 production. B. R. Rinn¹, A. K. Neff², G. M. Fincham², P. J. Kononoff², and A. L. Carroll^{*2}, ¹University of Utah, Salt Lake City, UT, ²University of Nebraska, Lincoln, NE.

Some feed additives that mitigate enteric methane (CH_4) production can result in an increase in hydrogen (H_2) production. When expelled to the environment, H_2 may intensify competition for hydroxyl radicals, prolonging CH_4 persistence in the atmosphere. Therefore, the objective of the experiment was to determine the effects of adding 2-bromoethanesulfonate (BE) alone and in combination with monensin (MON) or calcium propionate (CPro) on in vitro ruminal CH_4 and H_2 production. During 3 separate runs, 1 g of 50% corn silage and 50% alfalfa (DM basis) was fermented (1) alone (CON); (2) with 100 μM BE (BES); (3) BES + 2.5 μM MON (BM); and (4) BES + 2.5% DM CPro (BC). Inoculum was obtained by collecting a mixture of rumen fluid from 2 steers (BW = 657 ± 12.5 kg) consuming a diet of 30% concentrate and 70% roughage. For each treatment within run, gas production was measured over a 48-h period using 2 randomly assigned paired and separate modules. Total CH_4 and H_2 production was calculated by multiplying the total volume of gas produced by the concentration of CH_4 and H_2 using a gas chromatograph at 0, 4, 8, 18, and 24 h. Data were blocked by run and analyzed as a repeated measures randomized complete block design using the GLIMMIX procedure of SAS (9.4), where treatment, time and the interaction between these 2 factors were considered as fixed effects and module, run, and the interaction were included as random effects. Significance was declared at $P \leq 0.05$. The addition of BES, BM, and BC at 4 and 8 h did not change CH_4 production, averaging 0.30 and $0.70 \pm \text{mL}$ respectively, but CH_4 production was reduced compared with the CON (0.93 and 2.60 ± 0.250 mL CH_4 , respectively). At 8 h, BES increased H_2 production relative to BM and BC (0.908 and 0.636 ± 0.086 mL H_2 , respectively), but all treatments containing BES were significantly greater than CON (0.02 ± 0.086 mL H_2). Thus, results indicate that treatments containing BES reduced CH_4 , and the addition of CPro or MON may serve as a short-term strategy for the reduction of H_2 resulting from mitigation of enteric CH_4 production.

Key Words: in vitro, methane mitigation, hydrogen

2740 Evaluation of red algae sources on rumen gas production in vitro. A. Tiwari* and R. Kohn, *University of Maryland, College Park, MD.*

Identifying local products with ability to reduce methane production has been a key area of interest for researchers. Enteric methane mitigation has been inconsistent among species of algae due to various factors, including storing and processing methods. Therefore, the objective of this study was to compare the efficacy of 2 different sources of red algae on gas parameters in vitro. Trial 1 used self-harvested, oven-dried red algae (*Gracilaria* spp.), and trial 2 used a freeze-dried commercial

algae (*Asparagopsis taxiformis*). Fresh red algae were collected from Assateague Island, Maryland. Algae were washed with fresh water and oven-dried for 48 h at 60°C, and dried samples were ground through a 1-mm screen of a Wiley mill. Rumen fluid was collected from the same nonlactating Holstein Friesian cow on different days for each trial. In trial 1, 100% rumen fluid (20 mL) was used, whereas in trial 2, rumen fluid (30 mL) was mixed with buffered media (50:50). Timothy hay was used as substrate in both trial 1 (0.4 g) and trial 2 (0.76 g) with algae and without algae. Algae were included as 10% or 2% substrate DM in trials 1 or 2, respectively. Gas samples were collected at 10 min, 4 h, and 24 h and analyzed using gas chromatography. Differences between treatments were analyzed using ANOVA in JMP. Significant differences were accepted at $P < 0.05$. In trial 1, algae had no effect on gas parameters. Methane production was almost zero at all time points in trial 2. Cumulative gas at 4 and 24 h decreased by 6.3% and 7.6% in trial 2, $P < 0.05$. H_2 accumulation increased by 408%, 5,368%, and 52,152% at 10 min, 4 h, and 24 h in trial 2, and CO_2 production in trial 2 decreased by 5.4% and 2.8% at 4 and 24 h, $P < 0.05$. *Asparagopsis taxiformis* decreased methane production but also increased H_2 and decreased CO_2 , suggesting inhibition of digestion. *A. taxiformis* decreased methane production in vitro, but *Gracilaria* spp. did not. Source or harvesting method could affect efficacy of algae in decreasing methane production.

Key Words: antimethanogenic compound, fermentation, headspace gas

2741 Investigating the effects of feeding distillers grains to lactating dairy cattle on enteric methane emission metrics. K. C. Krogstad^{*1} and K. J. Herrick², ¹Department of Animal Sciences, The Ohio State University, Wooster, OH, ²POET, Sioux Falls, SD.

Reducing enteric methane emissions is a goal of the dairy industry as it strives to meet its net-zero emissions commitment. One avenue to reduce emissions associated with dairy farming is through feeding coproducts, such as corn-ethanol distillers grains (DG). Our objective was to conduct a mixed model meta-regression investigating the effects of DG supplementation on enteric methane emissions from lactating dairy cattle. We hypothesized that DG would reduce methane production, yield, intensity, and methane as a proportion of gross energy intake (Ym). We conducted a mixed model meta-regression with the fixed effect of DG and the random effect of study. All observations were weighted by the inverse of the standard error of the mean. We conducted subsequent analyses that included the effects of diet NDF and diet fat concentration. Significance was declared at $P \leq 0.05$, and trends were discussed at $0.05 < P \leq 0.10$. Seven experiments with 26 treatment means were collected for the analysis. Respiration chambers (2) or headbox style respiration chambers (5) were used to measure methane in these experiments. In models with the fixed effect of DG, methane production, yield, intensity, and Ym were all reduced as DG inclusion increased in the diet ($P \leq 0.001$). In models accounting for dietary fat and dietary NDF concentrations, DG inclusion reduced methane production, methane yield, tended to reduce methane intensity (ECM basis), and reduced Ym ($P \leq 0.08$). A best-fit model was then selected based on the model with the lowest Akaike information criterion (AIC); DG was forced into each model. The univariable model with DG inclusion had the lowest AIC for methane intensity (ECM basis) and Ym. The full model (DG + fat + NDF) yielded the lowest AIC for methane production. The DG + NDF model yielded the lowest AIC for methane yield and DG + fat yielded the lowest AIC for methane intensity (fluid milk basis). Inclusion of DG in diets for lactating dairy cattle reduces methane production, yield, intensity, and Ym, suggesting it may be a tool to reduce the GHG footprint of lactating dairy cattle.

Key Words: sustainability, coproduct, greenhouse gas

2742 Correlation between methane production and total-tract nutrient digestibility of dairy cows. M. R. Pupo^{*}, G. Martinez-Boggio, F. Peñagaricano, and L. F. Ferraretto, University of Wisconsin-Madison, Madison, WI.

Dairy cows contribute to climate change primarily through enteric methane (CH_4) emissions. However, selective breeding for reduced CH_4 production could affect total-tract nutrient digestibility. The aim of this study was to assess the correlation between CH_4 production and total-tract nutrient digestibility of multiparous dairy cows. Data were obtained from 113 lactating Holstein cows from 2 continuous lactation feeding trials (8–12 wk) conducted at the University of Wisconsin-Madison (2023–2024). Each study had 64 cows with assigned access to each of 32 electronic gate feeders and 2 GreenFeed units. Methane production (g/d) was measured using the GreenFeed system for the entire trial period and averaged by weighing visit duration, including only cows with at least 2 records per day and 3 d with records per week. Total-tract DM digestibility (DMD), OM digestibility (OMD), NDF digestibility (NDFD), and starch digestibility (TTSD) were determined using in situ 240-h undigested NDF (uNDF) as an internal marker. Six fecal grab samples were collected from each cow at 8- to 12-h intervals covering every 4-h clock period over 3 consecutive days during 2 wk of the treatment period. Total-tract digestibility of each individual nutrient was calculated from 240-h uNDF in situ and nutrient concentrations in theorts-adjusted diet and feces. Data were adjusted using a linear model that included cohort (trial and treatment: 8 levels) and week effects. The DMD, OMD, NDFD and TTSD averages ($\pm$ SD) were $71.8\% \pm 4.10\%$ DM (range: 57.5%–79.7% DM), $73.2\% \pm 3.96\%$ OM (range: 62.3%–81.3% OM), $48.2\% \pm 6.16\%$ NDF (range: 33.8%–61.4% NDF), and $99.0\% \pm 0.59\%$ starch (range: 97.0%–99.9% starch), respectively. The CH_4 production average was 426.3 ± 72 g/d, ranging from 243.0 to 658.7 g/d. The CH_4 production tended to be positively correlated ($P = 0.06$; $r = 0.14$) with NDFD. The CH_4 production was negatively correlated with DMD ($P = 0.05$; $r = -0.14$) and TTSD ($P = 0.01$; $r = -0.19$). However, CH_4 production was not correlated ($P = 0.15$; $r = -0.10$) with OMD. These findings suggest that cows with greater DM and starch total-tract digestibility might produce less CH_4 , but cows with greater NDFD produce more CH_4 .

Key Words: methane, efficiency, energy

2743 Effects of feeding a corn milling product and rumen-protected amino acids on methane emissions and milk production in lactating Holstein cows. S. C. Sherwood^{*1}, G. M. Fincham¹, A. L. Carroll¹, H. C. Wilson², H. E. Ohlde², and P. J. Kononoff¹, ¹Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE, ²OneTrak, Cargill Inc., Blair, NE.

The objective of this experiment was to determine the effects of feeding a corn milling product (CMP; OneTrak, Cargill Inc.) and rumen-protected amino acids (rpAA) on milk production and methane emissions. Sixteen multiparous Holstein cows (99 ± 18.2 DIM) were utilized in a quadruplicated 4×4 Latin square design with 4 periods of 28 d. Cows were blocked by milk production and randomly assigned to 1 of 4 treatments in a factorial arrangement: CON (no CMP, no rpAA); CONAA (no CMP, rpAA, including 0.37% lysine [Lys] and 0.11% methionine [Met]); CMP (CMP 40%, no rpAA); and CMPAA (CMP 40%, rpAA, including 0.53% Lys and 0.11% Met). Diets were formulated using NDS version 3.10.1.01 software assuming a DMI of 25 kg/d. The predicted supply of Lys and Met (Lys/Met g/d) supplied by metabolizable protein was 185 g/58 g, 206 g/74 g, 177 g/60 g, 209 g/76 g for CON, CONAA, CMP, and CMPAA respectively. Methane output was measured using headbox-style indirect calorimeters. Data were analyzed using the GLIMMIX procedure in

SAS (9.4) with fixed effects of treatment, square, and period nested in square and with random effect of cow nested in square. No interaction between CMP and rpAA inclusion was observed for any response variable ($P \geq 0.26$); thus, only main effects of CMP and rpAA are reported, with significant differences determined at $P \leq 0.05$. Production, yield, and intensity of methane output were reduced in diets containing CMP (635 vs. 530 ± 28.0 L/d; 24.8 vs. 22.1 ± 1.11 L/kg DMI; 16.5 vs. 13.4 ± 1.28 L/kg ECM respectively). Diets containing CMP increased milk yield (34.0 vs. 35.5 ± 1.33 kg/d). Milk protein percentage decreased in diets containing CMP but increased in diets with rpAA (3.29 vs. 3.15% $\pm 0.070\%$; 3.18 vs. 3.26% $\pm 0.070\%$, respectively). Treatments had no effect on milk protein yield, milk fat percent, milk fat yield, or ECM (average 1.12 ± 0.047 kg/d, 4.49% $\pm 0.162\%$, 1.56 ± 0.079 kg/d, and 40.1 ± 1.69 kg/d respectively). Results of this study indicate that feeding CMP significantly reduces enteric methane emissions without affecting milk fat or protein yield.

Key Words: corn milling coproduct, rumen-protected amino acid, methane

2745 Effects of dietary lipid supplements and feeding level on nutrient digestibility and methane emissions in dairy cows. F. A. Gutierrez-Oviedo^{*1}, M. J. Farricker¹, V. Ramos¹, M. You¹, A. Javai¹, V. R. Basnayake¹, N. D. Seneviratne², D. C. Reyes¹, J. R. Newbold², R. M. Kirkland³, and J. W. McFadden¹. ¹Department of Animal Science, Cornell University, Ithaca, NY, ²Dairy Research Centre, SRUC, Dumfries, United Kingdom, ³Volac Wilmar Feed Ingredients Ltd., Orwell, Hertfordshire, United Kingdom.

Fatty acids (FA) influence methane (CH₄) by altering organic matter dilution, microbial activity, and hydrogen (H₂) sinks. This study assessed the effects of 2 FA supplements on digestibility and CH₄ emissions. Thirty-six multiparous Holstein cows (138 $\pm$ 13 DIM, 43 $\pm$ 6.4 kg of milk/d) were assigned to a split-plot study design. The main plots included a palmitic acid-enriched supplement (HP) or calcium salts of palm FA (PO). Within each main plot, cows were assigned to one of 3 FA supplementation levels (0%, 1.5%, or 3% of diet DM) in a 3 $\times$ 3 Latin square design with three 21-d periods. Cows were fed a corn silage-based diet (14.3% CP; 31.4% aNDFom; 24% starch), where dietary FA replaced aNDFom. Samples were collected in the final 3 d of each period. Feed and feces gross energy was determined using bomb calorimetry, and ruminal CH₄ emissions were measured using GreenFeed (C-Lock, Inc.). Data were analyzed using a mixed model with fixed effects of plot, FA level, period, square, and plot $\times$ FA level, with cow nested within square as a random effect. The main plot effect was tested using cow (square) as the error term. Preplanned contrasts compared HP and PO at 1.5 and 3.0% FA levels; linear and quadratic contrasts were not tested due to constraints in the experimental design. CH₄ production (g/d) ($P = 0.02$), intensities (g/kg of milk or ECM) ($P < 0.01$), and yields (g/kg of DMI) ($P < 0.01$) decreased as FA levels increased, regardless of FA supplement. Digestibilities of DM ($P = 0.03$), OM ($P = 0.02$), and CP ($P = 0.001$) increased with FA dose. OM digestibility did not differ between cows fed HP and PO ($P = 0.51$), but aNDFom digestibility was higher for cows fed HP than PO ($P = 0.001$). Digestibility of EE was greater with PO than HP at 1.5% and 3.0% inclusion levels ($P < 0.01$). Gross energy digestibility did not differ ($P = 0.50$). CH₄ emissions, expressed as a percentage of gross energy intake, decreased with FA dose ($P = 0.01$). CH₄ emissions per kilogram of digestible OM were influenced by FA supplementation ($P = 0.58$), whereas CH₄ per kilogram of digestible aNDFom was not affected by FA product or dose ($P = 0.91$). These findings indicate that FA supple-

mentation influences nutrient digestibility and methane emissions, with differences observed between FA sources.

Key Words: fat supplement, energy, methane

2746 Grape seed tannin extract and unsaturated oils affect in vitro methane production and the microbiota to different extents. L. P. Thanh¹, V. T. P. Tien¹, T. T. T. Hang¹, N. Wichasit², Q. Jiang³, and J. J. Looor^{*4}. ¹Can Tho University, Can Tho, Vietnam, ²Naresuan University, Phitsanulok, Thailand, ³University of Illinois, Chicago, IL, ⁴University of Illinois, Urbana, IL.

Fish oil and condensed tannins in the ruminant diet could have synergistic effects on biohydrogenation (BH), CH₄ production, and the ruminal microbiota. To test these hypotheses, in vitro experiments were carried out as a completely randomized design with a control diet (CON) without added oil and grape seed tannin extract (GSTe, 95% proanthocyanidins), 2.5% soybean oil (SO), 2.5% SO and fish oil at 3:2 ratio (wt/wt; SFO), and SFO plus 0.6% grape GSTe (SFO-CT0.6). Ruminal contents used were from 4 lactating Saanen goats. Oils were emulsified with Tween-20 before incubations. Ruminal pH, NH₃-N, VFA, fatty acids, and digestibility were measured after 24 h of incubation. Cumulative total gas production and CH₄ concentrations were measured after 48 h. Data on ruminal fermentation, digestibility, and gas emission were analyzed with the GLM procedure in SAS OnDemand for Academics 2021 (SAS Institute, Inc., Cary, NC, USA). Fatty acid concentrations were analyzed using PROC MIXED with goat as a random effect and diet, time, and their interaction as fixed effects. DNA extracted from digesta was subjected to PacBio Full-Length 16S rRNA gene sequencing. High-quality amplicon sequence analyses were performed with Targeted Amplicon Diversity Analysis (TADA), MicrobiomeAnalyst, PICRUST2, and STAMP software. True digestibility was greater ($P = 0.04$) with SFO-CT0.6 than CON, but there were no effects of treatments on pH (6.70 $\pm$ 0.09), total (121 $\pm$ 30 mM) VFA, or NH₃-N (54.1 $\pm$ 11.8 mg/dL). Although total gas production (mL/g DM) did not differ, CH₄ (mL/g DM) was lowest ($P < 0.001$) with SFO-CT0.6 (23.9 mL/g DM) compared with CON (39.9 mL/g DM) and SO (37.2 mL/g DM). Compared with CON and SO, both SFO and SFO-CT0.6 led to lower ($P < 0.01$) 18:0 and greater vaccenic acid concentrations. Compared with other treatments, α -diversity Chao1 index was lowest ($P = 0.06$) with SFO-CT0.6, but the Shannon index did not differ. Treatments caused substantial effects on *Actinomyces*, *Synergistota*, and *Verrucomicrobiota* phyla, along with effects on *Coriobacteriia* and *Lentisphaeria* bacteria. Data indicate that the type of unsaturated oil and GSTe can have distinct effects on the ruminal environment.

Key Words: biohydrogenation, fermentation, metabolism

2747 Effects of dietary starch concentration and direct-fed microbial supplementation on lactation performance, feeding behavior, total tract nutrient digestibility, and enteric methane emissions by dairy cows. K. A. Juckem^{*}, M. R. Pupo, E. C. Diepersloot, and L. F. Ferraretto, Department of Animal and Dairy Sciences, University of Wisconsin-Madison, Madison, WI.

The objective of this experiment was to evaluate the effects of dietary starch concentration by replacing high-moisture corn with soy hulls and a direct-fed microbial containing *B. subtilis* 810 and *B. licheniformis* 809 (DFM) on lactation performance, feeding behavior, nutrient digestibility, and methane emissions of dairy cows. Sixty-four multiparous Holstein cows were randomly assigned to 1 of 4 treatments with a 2 $\times$ 2 facto-

rial arrangement for a 12-wk treatment period after a 1-wk acclimation period and 2-wk covariate period. Treatments consisted of diets with 21% starch and no DFM (RS-CON), diets with 21% starch and DFM (RS-DFM), diets with 27% starch and no DFM (NS-CON), and diets with 27% starch and DFM (NS-DFM). Data were analyzed as a completely randomized design using a mixed model including a covariate and dietary starch, DFM supplementation, week, and their 2- and 3-way interactions as fixed effects. Cow within treatment was a random effect. Reduced starch diets increased ($P < 0.05$) 1 kg/d DMI and 0.2% milk fat concentration but decreased ($P < 0.05$) feed efficiency by 0.07 kg milk/kg feed. Cows fed DFM tended ($P < 0.10$) to have 0.02% greater lactose concentrations. Milk yield and milk protein concentration did not differ ($P > 0.10$). Eating rate was 0.03 kg of DM/min lower and meal length 1.7 min/meal greater for DFM ($P < 0.05$). Cows fed NS had a shorter ($P < 0.05$) eating time (10 min/d), meal length (3.3 min/meal), and interval between meals (10 min) and a smaller meal size (0.4 kg DM). Meal frequency increased ($P < 0.05$) by 0.3 visits/d. Normal starch diets reduced ($P < 0.05$) methane emissions by 24 g/d, intensity by 0.4 g CH₄/kg ECM, and tended ($P < 0.10$) to reduce methane yield by 0.6 g CH₄/kg DM. Digestibility of DM, OM, CP, EE, and NDF was greater ($P < 0.05$) for DFM. The replacement of high-moisture corn with soy hulls increases milk fat concentration but not milk yield. Normal starch diets (27%) decrease methane production and improve feed efficiency compared with low starch (21%). Feeding cows DFM improved nutrient digestibility of dairy cows.

Key Words: starch, direct-fed microbial (DFM), production

2748 Effects of *Asparagopsis taxiformis* on the rumen microbiome of dairy cows. S. Heitman^{*1}, M. Sauzo¹, J. Felton¹, I. Salfer¹, A. Gomez¹, and B. J. Heins^{1,2}, ¹University of Minnesota-Twin Cities, St. Paul, MN, ²University of Minnesota-Morris, Morris, MN.

Methane continues to be a major problem the world faces. The potent greenhouse gas causes damage to our environment and atmosphere, coming from various sources, one being dairy farms. Dairy producers face restrictions on methane production but also understand the energy and efficiency loss from the cow in its production. Additive supplements, such as a red algae species (*Asparagopsis taxiformis*) containing bromoform with the brand name of SeaGraz, can inhibit methane production mainly by modifying the rumen microbiome, but there are still questions regarding its mode of action in the rumen microbiome, its long-term effectiveness, and potential side effects on animal productivity. To assess the effect of SeaGraz on the rumen microbiome, $n = 12$ cows were randomly assigned into 2 experimental groups (Control or SeaGraz) from September of 2023 to January of 2024. Rumen fluid was collected, and DNA was extracted and amplified targeting the V4 region of the 16S rRNA bacterial gene. Sequences were processed using bioinformatic tools, creating tables for counts of bacterial species within each sample. Results demonstrated a significant effect of SeaGraz on rumen bacterial composition ($R^2 = 0.086$, $F = 5.99$, and $P < 0.001$), including a significant effect of collection month ($R^2 = 0.31$, $F = 7.27$, $P < 0.001$) and an interaction between month and dietary treatment ($R^2 = 0.087$, $F = 2.03$, and $P = 0.005$). SeaGraz significantly reduced rumen bacterial diversity exhibited (Shannon diversity, $P < 0.001$) and species richness ($P = 0.001$) compared with the control group. Key methane-producing or aiding microorganisms, including certain species from *Bacilli* ($P = 0.001$), *Oscillospirales* ($P = 0.001$), and *Methanobrevibacter* ($P = 0.001$), were significantly reduced in the SeaGraz treatment, and their abundance also varied depending on the month sampled. In conclusion, SeaGraz supplementation significantly modulated the rumen microbiome and reduced the abundance of microorganisms potentially involved

in methane production. However, the effectiveness of SeaGraz may also vary month to month.

Key Words: methane reduction, rumen microbiome, dairy cow

2749 Effects of maternal dietary energy during late gestation in Holsteins on changes in growth, intake, and methane emissions of female offspring from birth until lactation. Z. Wu^{*1}, E. A. French², F. Peñagaricano¹, T. Cunha¹, L. L. Hernandez¹, and K. A. Weigel¹, ¹University of Wisconsin-Madison, Madison, WI, ²U.S. Dairy Forage Research Center, USDA-ARS, Madison, WI.

Maternal nutrient restriction or overabundance during late gestation has been associated with long-term metabolic and developmental consequences in offspring across various livestock species. This study evaluated the effects of maternal energy intake during late gestation on the growth performance, metabolic efficiency, and environmental effects of dairy heifers. A total of 80 multiparous Holstein cows were assigned to either a high-energy (HE) or low-energy (LE) diet, targeting 1.82 and 1.57 Mcal/kg of DM. Eighteen female calves from each treatment were monitored from birth through their first lactation. Growth parameters, including BW and body measurements (BM), were recorded at multiple time points. Additionally, enteric methane emissions were assessed using the GreenFeed system at 6, 12, and 18 mo of age, and during lactation measurements were conducted at 35, 65, 110, and 250 ± 10 DIM. No differences in BW or BM between treatment groups occurred throughout the study. Growth parameters were influenced by time ($P < 0.1$) but not maternal diet, with both HE and LE groups demonstrating comparable developmental trajectories. Similarly, methane emission analyses showed no significant differences ($P = 0.7$) in methane production (MP), residual methane intensity (RMI), or residual methane yield (RMY) between the 2 treatment groups from 6 to 18 mo of age, whereas MP was affected by the growth ($P < 0.1$). The Spearman rank correlation analysis indicated weak consistency in MP ranking across ages, with MP rankings showing minimal correlation between time points ($\rho = -0.03$ to 0.25). In contrast, RMY exhibited a slightly higher correlation at later stages ($\rho = 0.49$ between 12 and 18 mo), suggesting that RMY ranking fluctuates over time, particularly in early growth phases. These findings indicate HE and LE maternal diets during late gestation do not affect offspring growth or methane emission levels in dairy heifers. Further research is needed to assess potential effects on milk production efficiency and overall sustainability in dairy systems.

Key Words: lifetime efficiency, fetal programming

2750 The combined addition of disodium fumarate and fennel oil can inhibit rumen methane production. Y. Zhang^{1,2}, G. L. Li¹, Z. H. Wu¹, L. Ma¹, and D. P. Bu^{*1}, ¹State Key Laboratory of Animal Nutrition and Feeding, Institute of Animal Science (IAS), Chinese Academy of Agricultural Sciences (CAAS), Beijing, China, ²School of Life Science, Ningxia University, Yinchuan, Ningxia, China.

This study utilized an in vitro fermentation approach to investigate the effects of different combinations of disodium fumarate (DF) and fennel oil (FO) on methane production in the rumen. Rumen fluid was collected from 3 nonlactating rumen-cannulated Holstein cows with an average body weight of 650 ± 50 kg, which served as donors for the in vitro fermentation experiment. The study employed a full factorial design with 16 treatment groups established. Each group included 3 biological replicates, and the experiment was conducted twice for validation. The DF was supplemented at levels of 3%, 5%, and 7% (DF1, DF2, and DF3), whereas FO was included at 1.5%, 3%, and 4.5% (FO1, FO2,

and FO3). The control group contained only the substrate without any additives. All data were analyzed using SPSS 27.0 software. Compared with the control group, the levels of ammonia nitrogen ($\text{NH}_3\text{-N}$) were increased in groups FO3, DF1FO2, DF1FO3, DF2FO1, DF2FO2, and DF2FO3 ($P < 0.05$). Similarly, microbial crude protein (MCP) levels were increased in groups DF1, DF1FO1, DF1FO3, DF2, DF2FO1, DF2FO3, DF3, DF3FO1, and DF3FO3 ($P < 0.05$). Methane and carbon dioxide production were reduced in groups FO3 and DF1FO1 ($P < 0.05$), whereas the DF3 and DF3FO3 groups showed significant decreases only in methane production ($P < 0.05$). Additionally, the dry matter degradation rate of group DF2FO3 was lower than that of group control ($P < 0.05$). Overall, these findings suggested that fennel oil of 4.5% and the combination of 3% disodium fumarate with 1.5% fennel oil can effectively mitigate methane emissions.

Key Words: disodium fumarate and fennel oil, methane, rumen fermentation

2751 Seaweeds for cows fed canola meal or soybean meal: Effects on manure methane. C. Benchaar^{*1} and F. Hassanat², ¹*Agriculture and Agri-Food Canada, Sherbrooke Research and Development Centre, Sherbrooke, QC, Canada*, ²*Agriculture and Agri-Food Canada, Quebec Research and Development Centre, Quebec City, QC, Canada*.

The objective of this study was to evaluate the effect of replacing soybean meal (SBM) with canola meal (CM) without or with feeding seaweed to dairy cows on CH_4 emissions of stored manure. Twelve lactating cows were used in a randomized block design with a 2×2 factorial arrangement of treatments. Cows were fed (ad libitum, 5% orts) a TMR (52:48 forage:concentrate ratio on a DM basis) containing either CM or SBM (as the main protein source) without or with the addition (0.18%, DM basis) of seaweed (Synergraze Inc., Calgary, AB, Canada). Canola meal (25% of diet DM) was included in the TMR at the expense of SBM and soybean hulls. Manure (feces and urine) was collected from each cow (2 consecutive days), mixed (1:1) with an inoculum from a bioreactor, and incubated (20°C) under anaerobic conditions in glass bottles (3 replicates per cow) for 22 wk. The production and composition of gas were determined 4 times/wk. Manure was sampled to determine chemical composition at the end of incubations. Data were analyzed using the MIXED procedure of SAS to determine the effects of meal source, seaweed, and their interaction. Significance was declared at $P \leq 0.05$. No significant interactions between meal source and seaweed were observed for the amount of manure excreted or CH_4 and CO_2 emission. Volatile solid excretion was greater in cows fed CM than SBM (7.84 and 6.84 kg/d, respectively, SEM = 0.46) but was unaffected by feeding seaweed. Maximum production potential of CH_4 and CO_2 averaged 162 (SEM = 17.6) and 141 (SEM = 12.6) L/kg of volatile solids and was unchanged by meal source or seaweed. In conclusion, under the conditions of this experiment, no effect of meal source or feeding seaweed on CH_4 and CO_2 emission from stored manure was observed. However, cows fed canola meal excreted more volatile solids, which may contribute to increased CH_4 and CO_2 emissions in storage facilities.

Key Words: manure, methane emission, seaweed

2752 Methane emissions and production performance of cows fed a *Saccharomyces cerevisiae* fermentation-derived postbiotic. P. Piantoni*, Y. Roman-Garcia, I. Yoon, J. Davidson, and G. Schroeder, *Cargill Animal Nutrition and Health, Global Innovation Center, Elk River, MN*.

To evaluate the effect of a *Saccharomyces cerevisiae* fermentation-derived postbiotic (SCFP) on lactation performance and methane emissions of lactating cows, 26 Holstein cows (40.4 ± 6 kg/d milk yield; 221 ± 64 DIM; mean $\pm$ SD) were used in a randomized block design experiment with a 2-wk covariate and 6-wk treatment period. Treatments were a diet with or without SCFP at 19 g/d (NutriTek, Diamond V, Cedar Rapids, IA). The base diet was 20% forage NDF, 17% CP, and 29% starch. Cows were housed in individual tiestalls, and methane emissions were determined using the GreenFeed system (C-Lock Inc.) at 9 different time points between 0400 and 1900 h over the last 3 d of the covariate and treatment periods. Data from the last week on treatment were analyzed using the Lme4 package in R with a model containing the fixed effect of treatment, random effects of block and cow, and their respective covariate. The fixed effect of week and its interaction with treatment were added to the model to evaluate milk yield, DMI, and feed efficiency throughout the experiment. Supplementation of SCFP did not affect DMI (27.6 ± 0.62 kg/d) or feed efficiency (1.39 ± 0.04) throughout the treatment period. However, SCFP increased ($P_{\text{Treat} \times \text{Wk}} = 0.05$) milk yield after 2 wk of treatment and throughout the rest of the experiment by 1.5 kg/d on average. In addition, SCFP increased BW change at 6 wk (25.9 vs. 15.2 kg; $P = 0.01$). At 6 wk, SCFP tended to increase milk lactose yield (1.88 vs. 1.75 kg/d; $P = 0.09$) but did not affect yields of milk fat and true protein. In addition, SCFP decreased milk fat content (4.22 vs. 4.41%) and MUN (12.0 vs. 12.8 mg/dL; both $P < 0.05$) but did not affect milk protein and lactose content or SCC. Supplementation of SCFP did not affect ECM yield (44.0 vs. 41.9 kg/d; $P = 0.18$) and methane production (346 vs. 348 g/d), yield (12.6 vs. 13.1 g/kg DMI), or intensity (8.02 vs. 8.36 g/kg ECM; all $P > 0.40$) at 6 wk on treatment. Results indicate that the SCFP evaluated has the potential to increase milk yield and BW in mid- to late-lactation cows without affecting methane production, yield, or intensity.

Key Words: lactating cow, methane emission, postbiotic

2753 Production performance, neutral detergent fiber (NDF) digestibility, and the methane emissions of cows consuming diets containing different sources of copper sulfate. G. Ferreira* and P. Ghimire, *School of Animal Sciences, Virginia Tech, Blacksburg, VA*.

This study evaluated the production performance, the NDF digestibility (NDFD), and the methane emissions of cows consuming diets containing an unprotected copper sulfate (UCS) or a protected copper sulfate (PCS). Two mineral premixes were prepared in the laboratory to match the requirements of absorbable minerals of a 650-kg lactating cow producing 40 kg/d of milk. Soybean hulls were used as the mineral carrier. Cobalt sulfate, calcium iodate, zinc sulfate, manganese sulfate, ferrous sulfate, sodium selenite, and UCS were of reagent grade, whereas PCS (MaxxC60; Maxx Performance Inc.) and vitamins A, D, and E (DSM-Firmenich) were of feed grade. The 2 premixes were incorporated into 2 concentrates, and these (46%) were mixed with corn silage (40%), grass hay (9%), and triticale silage (5%). Twelve lactating Holstein cows (639 ± 45 kg BW and 210 ± 29 DIM) were housed in a freestall barn and individually fed using a Calan gate feeding system using a cross-over design with two 14-d periods. Milk yield and DMI were collected during the last 7 d of each period. Milk samples were collected during 4 consecutive milking sessions. Methane emissions were measured using a GreenFeed unit (C-Lock). The bait fed through the GreenFeed contained no minerals. Fecal grab samples were collected every 9 h during the last 3 d of each period (8 samples total). Undegraded NDF (uNDF) was used as an internal marker to estimate NDFD. Data were analyzed using the MIXED Procedure of SAS, and the model included the fixed effects of diet and period and the random effect of cow. Rela-

tive to UCS, feeding PCS did not affect MY (35.5 kg/d; $P = 0.85$), DMI (22.8 kg/d; $P = 0.27$), the milk fat (4.45%; $P = 0.14$), protein (3.44%; $P = 0.21$), and lactose (4.83%; $P = 0.56$) concentrations, the milk fat (1.56 kg/d; $P = 0.52$), protein (1.20 kg/d; $P = 0.49$), and lactose (1.71 kg/d; $P = 0.80$) yields, NDFD (34.8%; $P = 0.57$), methane production (375 g/d; $P = 0.97$), methane intensity (11.0 g/kg DM; $P = 0.83$), or methane yield (16.7 g/kg DM; $P = 0.59$). In conclusion, feeding a rumen-protected copper sulfate did not affect production performance, digestibility, or methane emissions.

Key Words: copper sulfate, NDF digestibility, methane

2754 Associations between fecal microbiome and methane emissions in lactating dairy cows. N. Sheybani*, A. P. M. Assumpção, G. Martinez Boggio, H. M. White, K. A. Weigel, H. C. Mantovani, and F. Peñagaricano, *University of Wisconsin-Madison, Madison, WI*.

Methane is the second-most abundant greenhouse gas and an important contributor to climate warming. Livestock, especially cattle, are widely recognized as a key source of methane emissions and major contributors to climate change. Beyond its environmental effects, methane also represents a loss of gross energy and, thus, inefficient use of feed. This study evaluated the associations between fecal microbiome and methane emission traits in lactating dairy cows. We measured methane production (MeP, in g/d) using GreenFeed, NEL, and BW in 704 midlactation Holstein cows from 20 different trials. Each trial lasted 8 wk, during which cows had continuous (24/7) access to GreenFeed for methane measurement. We calculated residual methane intensity (RMI) as the regression of MeP on NEL and metabolic BW, accounting for cohort effects as a combination of study and treatment. We collected fecal samples via rectum on the last day of each trial, and the fecal microbiome was assessed using 16S rRNA gene sequencing. We identified *Bacteroidota* (50%) and *Firmicutes* (44%) as the most abundant bacterial phyla, whereas *Euryarchaeota* (0.58%) was the most prevalent archaeal phylum in the fecal microbiome. Pearson correlation coefficients were used to explore the relationships between each taxon and methane production traits. We found several taxa, including *Methanobrevibacter*, *Bifidobacterium*, and *Methanosphaera*, positively correlated with MeP and RMI ($P < 0.05$). In contrast, *Prevotellaceae* and *Succinivibrionaceae* exhibited negative correlations with MeP and RMI ($P < 0.05$). Overall, our findings suggest that the fecal microbiome could be used as an easy-to-measure proxy trait for methane emissions in dairy cattle.

Key Words: 16S ribosomal RNA (rRNA) gene sequencing, microbial composition, gut microbiota

2755 Response of low and high methane-emitting Holstein Friesian cows to dietary interventions. H. Honerlagen*, L. Sebek², T. Wind², and S. van Gastelen², ¹*Department of Animal Breeding and Genomics, Wageningen University & Research, Wageningen, The Netherlands*, ²*Department of Animal Nutrition, Wageningen University & Research, Wageningen, The Netherlands*.

Dietary interventions are effective in mitigating enteric methane (CH₄) emission. However, variation in CH₄ emission exists within herds. The aim of this study was to determine (1) whether replacing grass silage (GS) with corn silage (CS) reduces CH₄ emissions equally effective in dairy cows with a naturally low or high CH₄ emission level, and (2) whether these cows remain low or high emitters irrespective of the diet fed. We measured the CH₄ emission of 192 lactating dairy cows fed the same diet using the GreenFeed system. Subsequently, we selected the 12 highest (23.6 g/kg DMI) and 12 lowest CH₄ emitters (16.3 g/kg DMI). The cows were blocked for parity, days in milk, and milk yield and were fed 2 diets in a crossover design: a GS-based diet with (DM basis) 58.3% GS, 19.4% CS, and 22.2% concentrate and a CS-based diet with 19.4% GS, 58.3% CS, and 22.2% concentrate. Treatment periods lasted 4 wk and consisted of 2 wk of adaptation followed by 2 wk of measurement. Diets were provided in feed intake recording bins. Concentrate was additionally fed in the milking parlor and in the GreenFeed system that was used to measure CH₄ emission. Data were analyzed using the lme4 package in R (version 4.4.2). The model included diet, group, and the interaction as fixed effects. For all analyses, the fixed effects of period and order of treatment were initially included in the model but removed because both were found not significant. The summarized results are in Table 1. The results indicate that (1) replacing GS with CS reduced CH₄ emission and was effective in both low (−38.0%) and high (−23.8%) CH₄-emitting dairy cows and that (2) dairy cows remained low or high CH₄ emitters irrespective of the diet fed (low CH₄ compared with high CH₄ was −19.1 and −34.2% for GS and CS, respectively). Yield of FPCM was not affected by diet or CH₄ emission level, but DMI was higher for CS compared with GS. For practical methane mitigation strategies, replacing GS with CS is effective irrespective of the variety in CH₄ emission levels within a herd.

Key Words: nutritional strategy, methane, cow emission level

2757 Effects of dietary lipid and cashew nutshell liquid supplementation on performance and ruminal methane emissions in dairy cows. V. R. Basnayake*, V. Ramos, L. A. Sprague, N. D. Senevirathne, and J. W. McFadden, *Department of Animal Science, Cornell University, Ithaca, NY*.

Dietary fat and functional oils effectively reduce ruminal methanogenesis. Our objective was to evaluate the interactive effects of feeding rumen-inert fat and cashew nutshell liquid (CNSL) with castor oil (CO) on milk production and enteric methane (CH₄) emissions in dairy cows. Thirty-six multiparous Holstein cows (2.7 ± 0.9 parity, 87 ± 29 DIM, 40 ± 5.3 kg milk/d) were used in a 4 × 4 Latin square design with four 21-d periods (16-d adaptation and 5-d sampling and data collection). Cows were fed a corn-silage and haylage-based diet (DM basis: 33% aNDFom and 23% starch) for a 21-d covariate period and then assigned to 4 treatment groups (n = 9): (1) basal TMR (CON); (2) CON with 12 g/d of CNSL and CO blend (CN); (3) CON with 2.0% (DM basis) fatty acids (C16:0; 51.47%, C18:0; 30.66%, C18:1; 8.78%, and C18:2; 1.88%) (FA); and (4) CN plus FA (CNFA). Data were analyzed using

Table 1 (Abstr. 2755).

Item	GS-based		CS-based		SEM	P-value		
	Low CH ₄	High CH ₄	Low CH ₄	High CH ₄		Diet (D)	Group (G)	D × G
CH ₄ yield (g/kg DMI)	24.5	30.3	15.2	23.1	1.45	<0.01	<0.01	0.46
Total DMI (kg/d)	16.2	16.9	23.6	22.2	0.96	<0.01	0.72	0.30
FPCM ¹ (kg/d)	33.8	29.7	33.9	32.1	1.32	0.51	0.12	0.54

¹Fat- and protein-corrected milk.

the MIXED procedure in SAS, with fixed effects for treatment, period, square, treatment $\times$ period, and treatment $\times$ square and a random effect for cow nested within square. Supplementation with FA decreased DMI ($P < 0.01$) and increased yields of milk and ECM relative to CON ($P < 0.01$). Consequently, feed efficiencies for MY and ECM were greater for cows fed FA relative to CON ($P < 0.01$). Higher milk fat and lactose concentrations ($P < 0.01$), albeit lower true protein concentrations ($P < 0.01$), were observed with FA relative to CON. Yields of milk fat also increased with FA ($P < 0.01$) relative to CON. Cows fed CNFA showed reduced BW ($P = 0.04$) relative to CON. Similarly, CNFA lowered BW gain ($P < 0.01$) relative to CON and FA. Ruminant pH and VFA concentrations, rumination, and physical activity were not affected by treatment. Supplementation with CNFA reduced CH₄ production (g/d) by 4.7% ($P < 0.01$) relative to CON. Methane intensities for MY and ECM were reduced for FA ($P < 0.01$) relative to CON. In conclusion, dietary fat feeding enhanced milk production to reduce methane intensity, whereas feeding CNSL with CO reduced methane production in dairy cows.

Key Words: methane, fat, functional oil

2758 Effects of the inclusion of Brominata (*Asparagopsis taxiformis*) on the performance of dairy cows. I. F. Carrari*¹, V. A. Achziger¹, E. F. Santos³, N. V. Tropia³, and M. I. Marcondes^{1,2}, ¹Washington State University, Pullman, WA, ²William H. Miner Institute, Chazy, NY, ³Universidade Federal de Viçosa, Viçosa, MG, Brazil.

Methane emissions from ruminants are a major environmental concern, and the seaweed *Asparagopsis taxiformis* has been suggested as a mitigation strategy, but its effects on animal performance need further studies. We evaluated the effects of Brominata pellets, a bromoform-stable fresh *A. taxiformis* product, on the dry matter intake (DMI), milk yield (MY), and CH₄, H₂, and CO₂ emissions of lactating Holstein cows. Twenty-eight lactating cows were enrolled in the experiment 2 d postcalving and housed in a freestall. The study included a 30-d adaptation period, after which the cows were assigned to 1 of 2 treatments: control or Brominata. The treatment phase consisted of a 7-d ramp-up period (50% of full dose) followed by 84 d of full-dose feeding. MY was measured twice daily during milking, and DMI was recorded daily using Callan Gates. CH₄, H₂, and CO₂ emissions were monitored daily using a GreenFeed system. Data were aggregated biweekly and analyzed using a randomized block design in a repeated measurement scheme. Results (Table 1) showed no significant differences in DMI and milk yield ($P > 0.05$). CH₄ (g/d) was significantly lower and H₂ (g/d) was significantly higher in the Brominata group ($P = 0.01$), and no differences were observed for CO₂ ($P = 0.28$). Additionally, CH₄/DMI and CH₄/MY (g/kg) were lower for the Brominata group, whereas feed efficiency (FE) was unaffected by treatment ($P = 0.63$). Our findings

Table 1 (Abstr. 2758). Effects of Brominata on dry matter intake, milk yield, and CH₄, H₂, and CO₂ production of lactating Holstein cows

Item	Treatment		SEM	P-values		
	CON	BROM		TR	BW	INT
DMI, kg/d	25.02	23.94	1.810	0.53	0.01	0.78
MY, kg/d	39.12	37.02	1.892	0.47	0.40	0.18
CH ₄ , g/d	386.92	208.71	36.682	0.01	0.01	0.39
H ₂ , g/d	2.38	9.44	0.286	0.01	0.92	0.81
CO ₂ , g/d	12,355	11,684	1,053	0.28	0.01	0.45
FE, kg/kg	1.57	1.54	0.050	0.63	0.01	0.16
CH ₄ /DMI, g/kg	15.72	8.91	1.073	0.01	0.01	0.32
CH ₄ /MY, g/kg	4.58	2.70	0.267	0.01	0.04	0.67

indicate that incorporating Brominata did not adversely affect DMI or MY but reduced CH₄ emissions, highlighting its potential as an effective methane-mitigating additive with sustained efficacy. These results suggest that Brominata can enhance the environmental sustainability of dairy production without compromising performance.

2759 Evaluating long-term methane-mitigating potential of a tank-grown *Asparagopsis taxiformis* variety (Brominata) in dairy cows. S. E. Omale and J. A. D. R. N. Appuhamy*, *Department of Animal Science, Iowa State University, Ames, IA.*

It is necessary to identify suitable *Asparagopsis taxiformis* (TA) varieties for land-based culture systems and to research the long-term CH₄-mitigating potential (CH₄MP) of these varieties. The study objective was to determine the long-term CH₄MP of a tank-grown AT variety, Brominata (BMT), in dairy cows. Forty-eight late-lactating cows were assigned to 4 dietary BMT inclusion rates (IR, n = 12): 0 (CTL), 0.30% (SIR), 0.45% (SIR1.5), and 0.60% of DM (SIR2.0) over a 4-wk IR ramp-up phase (P1) and then a 9-wk measurement period (P2). Freeze-dried Brominata was delivered in a pellet mixed into the TMR, and CH₄ emissions were measured using a GreenFeed unit. When analyzed with the MIXED procedure of SAS, CH₄ yield decreased linearly by 42%, 58%, and 60%, respectively, with increasing IR ($P < 0.01$), but the week $\times$ IR interactions ($P < 0.01$) indicated that CH₄MP changed with time differently in response to different IR. In the first week of P2, all IR had comparable percentage reductions of CH₄ yield from CTL (pCH₄Y; 77%). SIR1.5 and SIR2.0 sustained this pCH₄Y until wk 6 and showed a decrease to 39% as of wk 9, whereas the pCH₄Y of SIR began to decline right away and became negligible as of wk 9. In alignment with the diminishing CH₄MP, CHBr₃ intake (mg/d) decreased due to the decrease in BMT bromoform concentration (6.6 to 3.3 mg/g), and sorting against the pellets. The CHBr₃ intake did not explain the improved CH₄MP persistency of SIR1.5 and SIR2.0 versus SIR, as all IR had similar CHBr₃ intake during the first 6 wk. Perhaps the higher CHBr₃ intake (435 vs. 310 mg/d) during P1 exerted a more potent suppression of ruminal methanogens to result in a persistent CH₄MP and maintaining a certain level of CHBr₃ intake afterward was necessary to support that persistency. Each unit (mg/d) increase in CHBr₃ intake was related to a 0.23 $\pm$ 0.01 percentage point increase in pCH₄Y ($P < 0.01$; R² = 0.68) throughout P2. This efficacy coefficient for the last 5 wk was 24% lower than that of the first 4 wk (0.19 $\pm$ 0.01 vs. 0.25 $\pm$ 0.01), suggesting a CHBr₃ intake-independent component for the diminished CH₄MP.

Key Words: bromoform intake, microalgae

2760 Supplementation with a blend of essential oils, tannins, and bioflavonoids in dairy cows. II: Implications for methane emission reduction. J. G. Minutti*¹, F. Negreya⁴, M. Cabrera⁴, J. A. Echeveste⁴, J. Montini¹, J. I. Gere³, C. Cajarville², J. L. Repetto², and A. Santana¹, ¹Facultad de Veterinaria, San José, Uruguay, ²Sintesisnutricion S.L., Barcelona, España, ³Unidad de Investigación y Desarrollo de las Ingenierías UTN-FRBA-CONICET, Buenos Aires, Argentina, ⁴Facultad de Ciencias Agrarias UDE, Montevideo, Uruguay.

The study aimed to evaluate the effects of a specific blend of essential oils, tannins, and bioflavonoids (EOTB, ANAVRIN, Vetos Europe SAGL, Switzerland) on CH₄ emissions in dairy cows fed fresh forage (FF) combined with a partial mixed ration (PMR). Two treatments were evaluated using 16 multiparous Holstein $\times$ Jersey cows (57 $\pm$ 31 DIM) in a crossover design: (1) Control (PMR at 70% of potential DMI with 8 h unrestricted FF access), and (2) EOTB (Control diet + 3 g EOTB/cow per day) provided individually and mixed with half PMR allocation.

The experiment lasted 85 d, in 2 periods, with adaptation of 30 d and data collection of 12 d. Cows were weighed on d 0, 42, and 85 of the experiment. In each period, individual DMI of PMR and FF (19% DM, 23% CP, and 45% NDF) was estimated as the difference between feed offered and refused for 5 d. Cows were milked twice daily, with milk production recorded over 10 consecutive milkings. CH₄ emissions were quantified using the SF₆ tracer gas technique, with 5 consecutive days of collection, and CH₄ and SF₆ were analyzed by gas chromatography. Data were analyzed using the MIXED procedure of SAS. The model included the fixed effects of treatment, period, and treatment × period interaction and the random effect of the cow. In EOTB, the CH₄ (g/d), the emission rate (respect to BW^{0.75}), and emission intensity per kilogram of 3.5% SCM was reduced in 10%, 12%, and 14% respectively. These results suggest that the additive induced changes in rumen fermentation leading to enhanced nutrient utilization in animals receiving the supplement (Table 1). Our findings demonstrate a consistent positive effect of this blend of EOTB on reducing methane emissions.

Key Words: methane, phytogetic additive

2761 Methane emissions of pasture-fed dairy cows when fed a halogenated methane mitigant for an extended period. A. S. O Neachtain^{*1}, S. R. O. Williams¹, L. C. Marrett¹, R. Tognelli^{1,2}, M. Berkhout^{1,3}, S. S. Nair^{1,2}, C. Adam¹, S. Chandra⁴, and J. L. Jacobs^{1,3}, ¹Agriculture Victoria Research, Ellinbank, Victoria, Australia, ²Centre for Agricultural Innovation, School of Agriculture and Food, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Victoria, Australia, ³School of Applied Systems Biology, La Trobe University, Bundoora, Victoria, Australia, ⁴Agriculture Victoria Research, Tatura, Victoria, Australia.

Asparagopsis-based feed additives have shown antimethanogenic promise in recent years, being a natural source of the halogenated bioactive bromoform. Our study assessed the effects of feeding naturally derived bromoform from *Asparagopsis* spp. on enteric methane emissions of lactating dairy cows when fed over the course of an extended period (239 d). Sixty lactating, multiparous, Holstein-Friesian dairy cows (30.8 ± 5.1 d in milk, mean ± standard deviation) were randomly assigned 1 of 2 treatments following a randomized complete block design. Each treatment was incorporated into a molasses base and mixed with the animals' concentrate allocation twice daily: CON (0 bromoform per day) or BR (270 ± 27 mg bromoform per day, mean ± standard deviation). The basal diet consisted of *ad libitum* perennial ryegrass (*Lolium perenne* L.) pasture, along with supplemental pasture silage during late summer (experiment d 170 onwards). Methane emissions were measured at 3 time points by an SF₆ tracer technique: experiment d 35 to 39 (early lactation, EL); 109 to 113 (mid lactation, ML) and 235 to 239 (late lactation, LL). Methane production (g/d) was lowered by 14% in EL, 17% in ML, and 24% in LL in cows fed BR compared with

those fed CON (*P* < 0.001). Similarly, methane intensity (g/kg energy-corrected milk) was lowered by 13%, 14%, and 27% in EL, ML, and LL, respectively, when cows were fed BR (*P* < 0.001). There was no effect of BR on total milk yield or composition at any time point relative to CON. Samples of ruminal fluid were also taken concurrently with methane measurements. Although total volatile fatty acid concentrations (VFA) were largely unaffected, feeding cows with BR reduced the ratio of acetate to propionate in ruminal fluid samples by 9%, 8%, and 24% in EL, ML, and LL, respectively. We conclude that the addition of *Asparagopsis*-derived bromoform showed no loss of antimethanogenic potency when fed over a prolonged period and had no adverse effects on animal production.

Key Words: ruminant, greenhouse gas, *Asparagopsis*

2762 Dose-response effect of bromoform stabilized in vegetable oil on digestibility and methane production in an in vitro fermentation system. L. Gonzalez-Chappe*, A. Daudet, A. M. Pittaluga, and A. E. Relling, *Department of Animal Sciences, The Ohio State University, Wooster, OH.*

Bromoform (CHBr₃) is the primary compound responsible for the anti-methanogenic efficacy of red seaweeds. This study aimed to evaluate the effect of increasing CHBr₃ concentration in a bromoform-vegetable oil blend on digestibility and methane (CH₄) production in an in vitro fermentation system. Blends of CHBr₃ with vegetable oil were prepared to achieve formulated CHBr₃ concentrations of 0 (T0), 1,000 (T1), 2,500 (T2), and 5,000 (T5) mg/kg oil. All blends were included at 2% of the substrate (DM basis; diet contained 49% concentrate, 25% corn silage, 23% alfalfa haylage, and 3% grass hay). Ruminal contents were collected 2 h before the morning feeding from 2 ruminally cannulated Holstein cows. Buffered rumen fluid (60 mL) and the appropriate CHBr₃-oil blend were added to 150-mL vessels containing Ankom bags with 600 mg of the substrate and immediately placed in a shaking incubator at 39°C and 85 rpm for 3, 6, 12, 24, and 72 h of incubation (n = 9 per CHBr₃-oil blend and time, 3 blocks). After the incubation, fermentation was halted, and headspace gas samples were collected for CH₄ and carbon dioxide (CO₂) determination. Immediately after gas collection, pH and DM, NDF, and ADF disappearance were assessed. Data were analyzed using a mixed model considering the fixed effects of CHBr₃ concentration, incubation time, their interaction, and the random effect of block. There was no effect of the CHBr₃ concentration on pH, CO₂ production, or the disappearance rate of DM, NDF and ADF (*P* > 0.10). However, T2 and T5 reduced total gas production compared with T0 and T1 (*P* < 0.01). There was a CHBr₃ concentration × time interaction (*P* < 0.01) for CH₄ production. No differences were detected between treatments at 3 and 6 h. However, at 12, 24, and 72 h, T2 and T5 reduced CH₄ production compared with T0 (*P* < 0.01). At 72 h, CH₄ production was reduced by 96.4% and 99.5% in T2 and T5, respectively, compared with T0. Our

Table 1 (Abstr. 2760). Methane emitted by dairy cows fed fresh forage (FF) plus partial mixed ration (PMR) supplemented with a blend of essential oils, tannins, and bioflavonoids (EOTB)¹

Item	Treatment			P-value		
	Control	EOTB	SEM	Treatment	Period	Treatment × period
g CH ₄ /day per cow	381.8	344.5	12.35	0.04	0.58	0.46
g CH ₄ /kg BW ^{0.75} (emission rate)	3.3	2.9	0.13	0.05	0.67	0.21
g CH ₄ /kg DMI (emission factor)	16.7	15.5	0.58	0.14	0.02	0.28
g CH ₄ /kg SCM 3.5% (emission intensity)	12.5	10.7	0.69	0.03	0.41	0.57

¹Control: mixed diet without additive, EOTB: mixed diet supplemented with 3 g of ANAVRIN/cow per day; SCM = solid corrected milk at 3.5% = (0.4324 × milk yield kg) + (16.218 × fat kg).

results indicate that CHBr_3 stabilized in vegetable oil reduces in vitro methane production without negatively affecting feed degradability.

Key Words: bromoform, methane, seaweed

2763 Effects of combining *Asparagopsis taxiformis* and isoacids on enteric methane emissions and digestibility across 2 dietary forage levels. M. R. A. Redoy^{*1}, M. S. Bari¹, D. Kleinschmit², and M. E. Uddin¹, ¹Department of Animal Science, University of Connecticut, Storrs, CT, ²Zinpro Corporation, Eden Prairie, MN.

Asparagopsis taxiformis (AT) reduces enteric methane (CH_4) significantly but does not provide any cobenefits (e.g., productivity or gain) in dairy cows, whereas isoacids (ISO) enhance milk yield with modest CH_4 reduction depending on dietary forage level (FL), suggesting their combination may offer synergistic effects. Our objectives were to (1) evaluate 3 AT doses, 3 ISO doses, and their 9 blend combinations—a total of 15 treatments (TRT)—for their effects on enteric CH_4 and digestibility in high- (HF; 65%) and low-forage (LF; 51%) diets in Ankom RF system (Ankom Tech, USA), and (2) identify the optimal combination using a weighted scale, equally prioritizing DM digestibility (DMD) as a proxy for productivity and CH_4 reduction (50% weight for each). The AT doses were 10 (low), 20 (medium), and 30 (high) mg bromoform/kg DM, and ISO doses were 0.8 (low), 1.6 (medium), and 3.2 (high) g/kg DM. This experiment was designed as a randomized complete block (12 replicates/TRT), where each batch was considered a block. Rumen fluid was pooled from 3 Holsteins collected via esophageal tubing. Each culture bottle received 20 mL of rumen fluid diluted with 80 mL of buffer, along with 1 g of basal diet in a filter bag (F57, Ankom Tech., USA), and was incubated for 24 h in a shaking water bath at 39°C. Total gas production (TGP) was calculated from RF system, and CH_4 was quantified using gas chromatography. Data were analyzed using a mixed model with FL, TRT, and their interaction ($\text{FL} \times \text{TRT}$) as fixed effects and blocks as random effects. Our results showed that combinations (AT + ISO) increased TGP by 8% ($P < 0.01$) compared with AT alone but decreased it by 13% ($P < 0.01$) than ISO alone. Furthermore, AT+ISO produced a similar total CH_4 as AT alone but with levels 47% to 56% lower than that of ISO alone depending on FL ($\text{FL} \times \text{TRT}$; $P < 0.01$). In contrast, AT+ISO increased DMD by 5% ($P = 0.04$) compared with AT alone but decreased it by 13% ($P = 0.04$) compared with ISO alone. Similarly, AT+ISO tended to increase NDF digestibility by 7% compared with AT but decreased it by 8% than ISO alone under HF diet only (P

$= 0.07$). Finally, the weighted ranking indicated that AT (low) + ISO (medium) had the highest score in HF ($P = 0.02$), whereas AT (high) + ISO (medium) ranked highest in LF ($P = 0.04$). Overall, combining AT and ISO showed synergistic effects in reducing CH_4 and increasing DMD, where optimum combination depended on diet FL.

Key Words: feed additive, NDF digestibility, in vitro fermentation

2764 Flaxseed-based supplementation alters milk fatty acid profile without affecting milk yield or methane emissions in Holstein dairy cows. A. Akter^{*}, S. Wang, and E. Kebreab, University of California-Davis, Davis, CA.

Flaxseed, rich in polyunsaturated fatty acids (PUFA), is hypothesized to improve milk quality and reduce enteric methane emissions in dairy cows. This study investigated the effects of flaxseed-based feed supplementation on enteric emissions, milk yield, and milk composition in dairy cows. In a freestall barn, 24 multiparous Holstein dairy cows were assigned to a 3×3 Latin square design, receiving 3 treatments over 3 periods, each consisting of 4 wk. Treatments included a control diet (CON), a diet containing 4.5% LinPRO (4.5% LinPRO), and a diet containing 9% LinPRO (9% LinPRO) on a dry matter basis. GreenFeed was used to measure enteric emissions where cows had free access for the entire period. Alfalfa pellet bait was used to entice animals to GreenFeed. Weekly milk samples were analyzed for milk components, and fatty acid profiles were assessed from samples collected during the last 3 d of each period. Data were analyzed using mixed model ANOVA using PROC GLIMMIX in SAS 9.4. Dry matter intake did not differ among treatments. Enteric methane production was also unaffected by LinPRO supplementation (CON: 487 ± 10.1 g/d, 4.5% LinPRO: 488 ± 10.1 g/d, 9% LinPRO: 488 ± 10.1 g/d; $P > 0.05$). Similarly, milk yield, milk protein, lactose, SNF, and MUN did not differ among treatments ($P > 0.05$). However, milk fat percentage was reduced in both 4.5% LinPRO (4.47 ± 0.14) and 9% LinPRO (4.50 ± 0.14) groups compared with the CON group (4.67 ± 0.14). α -Linolenic acid, the predominant PUFA in flaxseed, was greater in the milk fat of LinPRO fed cows (1.05% and 1.46% in the 4.5% and 9% LinPRO groups, respectively) compared with that of cows in the CON group (0.52%). In conclusion, LinPRO supplementation did not influence methane emissions or milk yield but affected the milk fatty acid profile in dairy cows.

Key Words: flaxseed, methane emission, dairy cattle

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2765 Using a biennial blend as a replacement for barley silage in diets for midlactation dairy cows. I. M. Nyazika*, C. A. Bertens, K. S. Hare, H. A. Lardner, T. Mutsvangwa, and G. B. Penner, *University of Saskatchewan, Saskatoon, SK, Canada*.

The objective of this study was to evaluate the effects of increasing the dietary inclusion of a biennial blend silage (BBS) as a replacement for barley silage (BarS) on DMI, ruminal fermentation characteristics, apparent total-tract nutrient digestibility, and milk and milk component yields in lactating dairy cows. Eight ruminally cannulated multiparous Holstein cows (120 ± 44.4 DIM; 708 ± 69.4 kg BW) were used in a replicated 4×4 Latin square design with 28-d periods (18 d for dietary adaptation and 10 d for data and sample collection). Dietary treatments were a control diet (CON) containing 43.0% BarS (DM basis) as the sole forage or diets containing 14.1 (LOW), 28.1 (MID), or 43.0% (HIGH) BBS by replacing for BarS. The BBS was composed of triticale, sweet clover, red clover, and hairy vetch. Data were analyzed using the GLIMMIX procedure of SAS v9.4 to evaluate the linear, quadratic, and cubic effects of increasing BBS inclusion. Fixed effects included treatment, period and square, whereas cow within square was included as a random effect. As the inclusion of BBS increased, apparent total-tract ash-corrected NDF digestibility increased from CON to MID and then decreased for HIGH (quadratic effect, $P = 0.01$). Increasing BBS inclusion linearly decreased apparent total-tract DM digestibility but had no effect on starch or crude protein digestibility. Milk (quadratic, $P = 0.04$), fat (quadratic, $P = 0.03$), and protein (quadratic, $P = 0.04$) yields increased from CON to MID and then decreased from MID to HIGH. Increasing the inclusion rate of BBS as a substitute for BarS in dairy cow diets reduced DMI, whereas moderate inclusion rates (14.1 to 28.1%) improved milk, fat, and protein yields, likely due to the higher fiber digestibility of BBS.

Key Words: barley silage, biennial blend, dairy cow

2766 In vitro assessment of different types and fractions of colostrum as an additive to modulate rumen microbial fermentation after weaning. J. Muñoz-Grein, M. Fondevila, M. A. Latorre, and A. Belache*, *Dep. Producción Animal y Ciencia de los Alimentos, Universidad de Zaragoza, Zaragoza, Zaragoza, Spain*.

Colostrum supplies essential fats, proteins, carbohydrates, immunoglobulins (Ig), and bioactive compounds for neonatal nutrition and passive immunity. Additionally, it may enhance rumen function when supplemented during weaning. This in vitro study evaluated 3 colostrum types: ovine (OC), bovine (BC), and reconstituted BC (RBC). Each was fractionated sequentially into 4 components: whole colostrum (F1), fat-free colostrum (F2), Ig-free colostrum (F3, filtered at 100 kDa), and protein-free colostrum (F4, filtered at 10 kDa). Each fraction was tested at 3 supplementation levels (0%, 0.4%, and 2% vol.). Rumen inocula from 4 postweaning, artificially reared lambs were used as experimental units. Incubation was conducted in batch cultures using a concentrate-rich diet, and fermentation parameters were measured after 24 h of incubation at 39°C. Analysis of variance tested dose effects within each fraction and fraction effect within each doses, with lambs treated as a random effect. Results showed similar effects for the 3 types of colostrum. Dietary supplementation with 2% of colostrum (independent of the fraction) increased the rumen fermentation in terms of gas production ($P < 0.05$) and methane output ($P < 0.05$), alongside a reduction in pH ($P < 0.05$). Total VFA concentration was unaffected

by the treatments, but supplementation with F1 and F2 increased the branched-chain VFA ($P < 0.05$) and ammonia-N concentrations ($P < 0.001$), suggesting enhanced proteolysis. Supplementation with F1 and F2 promoted a decrease in the propionate molar proportion ($P < 0.05$) in both ovine and bovine colostrum. In conclusion, dietary supplementation with either whole homologous (OC) or heterologous colostrum (BC and RBC) during the postweaning period could boost the microbial rumen fermentation through the presence of different bioactive compounds, such as lipids, Ig, lactoferrin, lactose, and small peptides.

Key Words: batch culture, colostrum, rumen microbial fermentation

2767 Effects of zinc hydroxy trace mineral source on 48-hour in vitro fermentation and CH₄ production. C. B. Peterson*, K. F. Maldonado¹, J. S. Heldt¹, K. E. Griswold¹, and J. Johnston², ¹*Selko USA, Indianapolis, IN*, ²*Fermentrics Technologies Inc., Arnprior, ON, Canada*.

The present study was designed to understand the effect of zinc hydroxy trace mineral source on 48 h in vitro fermentation parameters. The supplemental trace minerals (STM) were calculated to provide 750 Zn in mg/d for a lactating dairy cow consuming 25 kg DM with a 120-L rumen volume. STM levels were scaled to fit a 250-mL fermentation vessel containing a mix of KSU buffer (80%) and rumen fluid (20%) with 400 mg of 6-mm ground TMR inside 50 ± 10 -µm Ankom bags. The experimental unit (vessel) was incubated for 48-h in a 39.5°C insulated water bath. Each treatment was replicated 6 times and included the following: Control (C, no additional feed additive), IntelliBond Zn (IBZ, 55% metal), IntelliBond Zn High Concentrate (IBZHC, 60% metal), sulfate zinc (SZ), and Availa zinc (AZ). Measurements included apparent microbial biomass production (aMBP, mg/g), apparent organic matter disappearance (aOMD, %), and methane (CH₄, mL). Data were analyzed as a complete randomized design one-way ANOVA, with multiple mean comparisons done with Tukey's HSD (JMP 18.1, SAS, Cary, NC). Significance was set at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.20$. For aMBP, there was a significant effect of treatment ($P = 0.0035$). The least squares means of the treatments were 153.3 C, 148.92 IBZ, 156.5 IBZHC, 150.2 SZ, and 158.75 AZ. Additionally, aOMD was significant ($P \leq 0.001$) with the AZ, IBZ, and IBZHC, (61.39, 59.96, and 58.13, respectively), not significantly different (NSD) from C (57.29), but significantly greater than the SZ treatment (54.4). Methane at 20 h was significantly affected by treatment ($P = 0.0028$) with C (10.01), and IBZHC (9.34), significantly higher than SZ (6.97) but NSD from AZ (9.203), and IBZ (7.82). Zinc source has a direct effect on fermentation patterns with sulfate Zn having a consistently negative effect.

Key Words: hydroxy trace mineral, fermentation

2768 Effects of NDF and mineral source on 48-h in vitro fermentation. C. B. Peterson*, K. F. Maldonado¹, J. S. Heldt¹, K. E. Griswold¹, R. Johnston², and J. Johnston², ¹*Selko USA, Indianapolis, IN*, ²*Fermentrics Technologies Inc., Arnprior, ON, Canada*.

A study was conducted on the interaction of neutral detergent fiber digestibility (NDFd) and mineral source on in vitro fermentation. Supplemental trace minerals (STM) were calculated to provide 125 Cu, 500 Mn, and 750 Zn in mg/d for a lactating dairy cow consuming 25 kg of DM with a 120-L rumen volume. The levels of STM were scaled to

fit a 250-mL fermentation vessel containing a mix of KSU buffer (80%) and rumen fluid (20%) with 400 mg of 6-mm ground TMR inside 50 $\pm$ 10- μ m Ankom bags. The experimental unit (vessel) was incubated for 48-h in a 39.5°C insulated water bath. The trial was arranged as a 2 $\times$ 3 factorial with 2 levels of NDF and 3 sources of trace mineral (TM; IntelliBond, sulfate, no additional mineral). Differences in NDFd were accomplished using BMR (high digestibility) and conventional (low digestibility) corn silage. Each treatment was replicated 6 times and included the following: high digestibility (HD) $\times$ control (HC, no additional mineral), poor digestibility (PD) $\times$ control (PC, no additional mineral), HD $\times$ IntelliBond (HI), PD $\times$ IntelliBond (PI), HD $\times$ sulfate (HS), and PD $\times$ sulfate (PS). Measurements included apparent microbial biomass production (aMBP) and apparent organic matter disappearance (aOMD). Data were analyzed as a 2-way ANOVA with a 2 $\times$ 3 factorial arrangement, with multiple mean comparisons done with Tukey's HSD (JMP 18.1, SAS, Cary, NC). Significance was set at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.20$. For aOMD, there was no significant differences (NSD) for the interaction of NDF $\times$ TM ($P = 0.5328$), but the main effect of both NDF and TM was significant ($P < 0.05$). The means of the treatments were 45.40 PI, 43.52 PC, 37.66 PS, 65.88 HI, 60.25 HC, and 57.11 HS. For aMBP, NDF $\times$ TM was significant ($P = 0.022$), with 107.83 PI, 107.38 HC, 104.67 HI, and 96.22 PC being significantly greater than the PS at 83.42 and NSD from the HS at 96.08. Both NDF and STM source are responsible for changes in in vitro fermentation.

Key Words: NDF, hydroxy trace mineral, fermentation

2769 Effects of rumen- and postrumen-targeted feed additives on in vitro digestion and microbial protein production. C. B. Peterson^{*1}, K. F. Maldonado¹, J. S. Heldt¹, K. E. Griswold¹, R. Johnston², and J. Johnston², ¹*Selko USA, Indianapolis, IN*, ²*Fermentrics Technologies Inc., Arnprior, ON, Canada*.

Objectives were to evaluate rumen- and postrumen-targeted feed additives (FA) on digestion and microbial protein production in an in vitro fermentation system (Fermentrics, Inc.). Treatments calculated for a lactating dairy cow with 25 kg of DMI and a 120-L rumen volume were scaled to fit a 250-mL fermentation vessel containing an 80:20 mix of Kansas State University buffer and rumen fluid with 400 mg of ground TMR inside 50- μ m Ankom bags. Vessels were incubated for 48 h at 39.5°C. Each treatment had 6 replicates. Treatments were as follows: Control (C, no feed additive); Renergy Original (RO) and Renergy HC (RHC) as rumen-targeted FA; and Fytera Lacteco (FL), Fytera Advance (FAD), and LactiBute (LB) as postrumen targeted FA. All FA were provided by Selko USA, Indianapolis, IN. Measurements included apparent microbial biomass production (aMBP) and apparent organic matter disappearance (aOMD). Apparent microbial biomass production is the main driver of milk yield estimation in the Fermentrics system. Data were analyzed as a complete randomized design by one-way ANOVA, with multiple mean comparisons done with Tukey's HSD (JMP 18.1, SAS, Cary, NC). Significance was set at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.20$. For aMBP, the RO treatment (159.9) was significantly greater ($P = 0.04$) than the FL treatment (131.7), but RO and FL treatments were not significantly different from the remaining treatments, including the control (FAD 157.4, RHC 155.8, LB 153.8, C 148.4). For aOMD, the RO, LB, and RHC treatments (44.5, 44.4, 43.8, respectively) were significantly greater ($P < 0.01$) than the C and FL treatments (37.3, 37.2) but were not significantly different from FAD (40.201). Overall, for the in vitro fermentation system used in this study, the tested rumen- and postrumen-targeted feed additives had mixed effects on digestion and microbial protein production.

Key Words: phytogetic, fermentation, rumen

2770 Grind size and method affect rumen digestibility in commercial analysis. E. M. Coons^{*1} and J. P. Goesser^{1,2}, ¹*Rock River Laboratory, Watertown, WI*, ²*Department of Animal and Dairy Sciences, University of Wisconsin-Madison, Madison, WI*.

This project objective was to determine if laboratory rumen digestion methodology affected dry matter (DMD) and starch digestion (SD). Five feed types (corn silage, cracked corn, high moisture corn, sorghum silage, and millet/corn) were split and left unground or ground to pass 6-mm, 4mm, or 1-mm sieves. Samples for in situ (IS) digestion (ISSD) were classified as coarse (unground), medium (6 mm), or fine (4 mm). Samples for in vitro digestion were also classified as coarse (6 mm), medium (4 mm), or fine (1 mm) either with (IVF) or without (IV) residue filtering before starch content measures. All samples were assayed in 3 rumen digestion runs, with 2 subsamples per hour, and were incubated for 3, 7, or 24 h. The in vitro digestion protocol used rumen fluid pooled from 3 cows. After in vitro incubation, IVF samples were filtered through paper (15 μ m), dried, and weighed for DMD and SD measures. For IV, residue starch concentration was determined within the in vitro rumen solution directly. For ISSD, 3 g of sample were weighed into R510 (50 μ m) Dacron bags and digested. Following ISSD, bags were rinsed in cold water, dried, and weighed to determine DMD, and then residues were compiled and assayed for starch to determine SD. Data were analyzed using the model fit function and linear mixed model approach JMP. Method, run, and coarseness were fixed effects, and feed type was treated as random. Interactions were evaluated. For this article, the 7-h data are analyzed and presented as this measure was most common among commercial dairy samples. Rumen 7-h DMD was greater for IS than IVF treatments (43.8% vs. 30.3%, $P < 0.001$). DMD was also greater for more finely processed samples (45.4% fine, 37.4% medium, and 28.3% coarse, $P = 0.02$). Seven-hour SD was affected by method, with IVF and IS being greater than IV (66.6% and 58.5% vs. 42.6% DM, $P < 0.001$). For 7-h data, SD was not affected by grind size or run, which warrants further evaluation. Based upon these results, methodology clearly affects both 7-h DM and SD. These results are in alignment with prior results. Further, these results can be used in dairy diet modeling efforts for relating data from different commercial SD analysis techniques.

2771 Ruminal fermentation in lactating Holstein and Jersey cows fed diets with differing levels of alfalfa or corn silage. G. I. Zanton¹, G. L. Menezes², M. B. Hall^{*1}, and J. R. R. Dorea², ¹*U. S. Dairy Forage Research Center, USDA-Agricultural Research Service, Madison, WI*, ²*Department of Animal and Dairy Sciences, University of Wisconsin-Madison, Madison, WI*.

The objective of this study was to evaluate the effects of alfalfa or corn silage-based diets on ruminal responses in rumen-cannulated, lactating Holstein (H) and Jersey (J) cows. Six H (mean $\pm$ SD: 194 $\pm$ 49 DIM, 661 $\pm$ 32 kg BW, 45.8 $\pm$ 3.78 kg milk/d) and 6 J (190 $\pm$ 37 DIM, 451 $\pm$ 33 kg BW, 26.0 $\pm$ 7.51 kg milk/d) cows were individually fed treatment diets across two 28-d periods according to a crossover experimental design. Diets were formulated to contain 22% forage NDF with 40% of dietary DM coming from corn silage and 20% from alfalfa silage (CS) or 40% of dietary DM coming from alfalfa silage and 20% from corn silage (AS), with concentrate ingredients adjusted to maintain CP (17%) and starch levels (26%). Over d 18 to 28 in each period, water intake, DMI, and milk yield were measured; milk was collected for compositional analysis; rumen fluid was sampled at 10 time points in 24 h; rumen contents were evacuated; and saliva was collected from the cardia. Data were analyzed as a split-plot, crossover design with the effects of breed, diet, and their interaction evaluated. Significance was

declared at $P < 0.05$ and tendencies at $0.05 \leq P < 0.10$. Breed affected water intake, DMI, milk yield, and composition as expected. Cows fed CS had higher DMI and milk protein yield and content. Saliva flow per DMI was unaffected by breed but tended to be higher for AS (12.4 ± 1.26 L/kg) than CS (10.5). Minimum ruminal pH was higher for J (5.99 ± 0.049) than H (5.74), and average pH tended to be higher for J than for H; no effect of diet was detected. Ruminal VFA concentration tended to be higher in H (122.3 ± 2.21 mM) than J (115.2), and ruminal NH_3 was higher in AS (3.45 ± 0.136 mg/dL) than CS (2.79). As molar proportions, cows fed AS had higher acetate than CS, whereas cows fed CS had higher propionate than AS across both breeds; J had higher butyrate than H across both diets. Mass of rumen solids and fluid were unaffected by diet but were higher in H than J; J had a higher proportion of rumen contents that were fluid ($55.2 \pm 1.21\%$) than H (50.0%). Diet and breed independently and without interaction affected various ruminal characteristics measured in this study.

Key Words: rumen fermentation, breed, forage

2772 Finishing of F1 Holstein cattle in northern Mexico with a ruminal fermentation additive in the diet and its effects on performance and beef grading. E. Carrillo-Moreno^{*1}, E. Carrillo², E. Perez-Reboloso^{1,3}, M. Mellado¹, D. Carrillo-Moreno¹, and F. G. Veliz¹, ¹Universidad Autónoma Agraria Antonio Narro, ²Instituto Tecnológico de Torreón, ³Centro de Bachillerato Tecnológico Agropecuario N°47.

The objective of this study was to evaluate the effects of a ruminal fermentation additive (EfficienZ, an exogenous enzyme preparation) on performance parameters and beef carcass grading of F1 Holstein/beef cattle during the finishing stage (>300 d of age). Animals were fed the same diet, but one group was supplemented with a ruminal fermentation additive (EfficienZ, Eff-z group) or with no additive (control). For this study, 80 male F1 cattle were completely randomly divided (based on weight, age, and average daily gain) into 2 groups (40 n/group), Eff-z or control. Data were analyzed by ANOVA, using the Fit Model procedure of JMP 18 Pro (SAS Institute Inc., Cary, NC). The effect of the ruminal fermentation additive was observed in the fecal starch content as determined by NIRS, which was lower ($P < 0.02$) in the Eff-z group ($3.07\% \pm 1.0\%$ vs. $6.14\% \pm 1.04\%$) than the control group, and the apparent total digestibility of starch in the digestive tract (TTSD) was higher ($P < 0.02$) in the Eff-z group ($98.05\% \pm 0.16\%$ vs. $95.78\% \pm 0.53\%$) than the control group. This resulted in the improvement of performance parameters: the final weight of animals in the Eff-z group was 13.57 kg higher ($P < 0.05$) than those in the control group (542.30 ± 22.24 vs. 528.73 ± 22.68 kg). In the age at sale, no statistical differences were observed ($P > 0.6$), but a reduction of 3.27 d in age at sale was observed in the Eff-z group (427.43 ± 27 vs. 430.70 ± 29 d). In terms of beef carcass grading (automatic value by VBG 2000), the Eff-z group had 12.50% more animals classified as choice than the control group (87.5% vs. 75.0%), the ribeye area was larger in the Eff-z group ($P < 0.08$) than in the control group (12.44 ± 0.48 vs. 12.26 ± 0.43 cm), and ADG was higher ($P < 0.01$) in the Eff-z group (1.273 ± 0.21 vs. 1.104 ± 0.20 kg) than control group. In conclusion, this study demonstrates the benefits of using a rumen fermentation additive (EfficienZ) in a finishing cattle feeding program on performance and carcass classification of F1 Holstein/beef cattle and describes how the additive promotes feed efficiency and contributes to improving the profitability of the dairy industry.

Key Words: beef on dairy, starch, beef grading

2773 Effects of periparturient supplementation of prototype postbiotics on blood metabolic profile and liver enzymes in transition dairy cattle. S. Saikumar^{*1}, K. Arriola¹, A. A. Vargas¹, M. C. Perdomo¹, M. U. Siregar¹, N. Alfaro-Diaz¹, A. Barahona¹, S. Salvatierra¹, A. Cayetano¹, I. Yoon², and D. Vyas¹, ¹University of Florida, Gainesville, FL, ²Cargill Animal Nutrition, Wayzata, MN.

This study aimed to evaluate the effects of supplementing prototype postbiotics on the blood metabolic profile and liver enzymes during the periparturient period (−35 to +63 d postpartum). We used a randomized block design with 62 multiparous dry Holstein cows ($\sim 245 \pm 3$ d pregnant, BCS 3 ± 0.29) assigned to 1 of 2 groups: control (CON; $n = 31$) or postbiotic prototype (PB; $n = 31$). Cows were blocked by parity, calving date, and previous 305-d milk yield (average: 47.5 kg/d). Diets were formulated according to CNCPS guidelines, and PB was top-dressed (25 g/d) on the total mixed ration. Blood samples were collected on d −35, −28, −14, −7, +3, +7, +14, and +21 relative to calving, as metabolic changes are most pronounced around 3 weeks pre- and postcalving. Prepartum and postpartum data were analyzed separately using the GLIMMIX procedure in SAS and d −35 values as covariates. Fixed effects included treatment, time, and their interaction, and cows nested within treatments were considered random factors. Significance was declared at $P \leq 0.05$, with tendencies noted at $0.05 < P \leq 0.10$. Milk yield was 52.7 vs. 51 kg/day for PB and CON respectively ($P = 0.06$). Greater levels of prepartum gamma-glutamyl transferase (GGT; $P < 0.01$; 26.5 vs. 22.9 ± 0.85 U/L) and blood urea nitrogen (BUN; 18.3 vs. 16.1 ± 0.62 mg/dL; $P = 0.02$) were observed with PB. Alkaline phosphatase (ALP) tended to be lower with PB on d −7 (treatment $\times$ day; $P = 0.06$). Postcalving, the albumin-to-globulin ratio was greater with PB supplementation (1.25 vs. 1.14 ± 0.07 ; $P = 0.01$). Calcium concentrations were lower on +14 and +21 d with PB supplementation (treatment $\times$ day; $P = 0.03$). No treatment effects were observed on glucose (prepartum: $P = 0.31$; postpartum: $P = 0.61$) or nonesterified fatty acids (prepartum: $P = 0.60$; postpartum: $P = 0.18$). In conclusion, postbiotic supplementation had limited effects on blood metabolites but notable effects on GGT, BUN, ALP and the albumin-to-globulin ratio, suggesting potential influences on liver function, protein metabolism, and immune status in transition dairy cows.

Key Words: postbiotic, transition cow, blood profile

2774 Buckingham-pi theorem for a dual-flow continuous culture system similarity evaluation. A. Mansouri^{*2}, M. Johnson¹, G. Leath², C. Rachid², A. Harding², A. Faciola¹, and M. Traum², ¹Department of Animal Sciences, University of Florida, Gainesville, FL, ²Department of Mechanical & Aerospace Engineering, University of Florida, Gainesville, FL.

Dual-flow continuous culture (DFCC) is an established in vitro rumen fermentation simulation technique. Commercial DFCC are rarely available, requiring researchers to build their own, which introduces variations that may compromise comparability. To mitigate this, the Buckingham-pi theorem from engineering fluid mechanics creates a framework across DFCC by grouping critical variables into dimensionless terms. If terms remain constant across systems, the similarity condition, empirical functions from standard DFCC predict performance of any other DFCC. Based on experience at the University of Florida (UF), 10 key variables are recommended: digesta density (ρ), viscosity (μ), specific heat capacity (c), temperature (T), acidity (pH), vessel diameter (D), vessel fill height (L), impeller diameter (d), impeller angular velocity (ω), and impeller power (P). The variable space spans 4 orthogonal reference dimensions, and the Buckingham-pi theorem imposes 6

dimensionless groups to characterize any DFCC. To experimentally validate this assertion, a bespoke DFCC was built with enhancements including (1) directly driven impellers, (2) a heated immersion water bath, and (3) digital data acquisition and output. A comparative experiment was conducted at UF's Animal Sciences Department operating 8 DFCC under identical feeds and protocols. The bespoke unit ran in parallel maintaining near geometric, kinematic, and dynamic/thermal similarity across 5 of the 6 dimensionless pi terms (L/D , d/D , $D^2\omega\rho/\mu$, $D^2T\omega^2/\mu$, and pH). Power number ($P/\rho\omega^3d^5$) was not matched owing to motor dissimilarities between the old and new units. Outputs—metabolic, biological, and chemical—were analyzed. The mean pH difference in 19 periodic measurements over 2.5 d between the DFCC aggregate and bespoke unit was 0.259 ± 0.057 (at a 95% confidence level). Measured digesta ammonia concentrations measured at 1-, 2-, 4-, 6-, and 8 h post-feeding showed comparative biological agreement. These findings give preliminary confidence that new DFCC adhering to Buckingham-pi similarity produce results consistent with traditional DFCC. This initial result shows promise for enabling broader ruminant nutrition research without requiring modifications to existing standards.

Key Words: dimensionless analysis, fluid mechanic, rumen fermentation

2775 Effects of high-protein duckweed on total-tract apparent digestibility in sheep. N. Stepanchenko^{*1}, Y. Kim¹, C. J. Eifert¹, A. Richards¹, C. V. Almeida¹, S. Issabekova¹, R. Brennan², and A. N. Hristov¹, ¹Department of Animal Science, The Pennsylvania State University, University Park, PA, ²Department of Civil and Environmental Engineering, The Pennsylvania State University, University Park, PA.

Duckweed (DW) is a small aquatic plant with nutrient absorptive properties that result in high protein (CP) concentrations when grown in nutrient rich water. The objective of this experiment was to investigate the effects of 0% (CON, basal diet comprised of 87.6% grass hay, 8.4% canola meal, and 4% molasses), 5% (LDW), and 10% (HDW) DW inclusion (on DM basis, replacing canola meal) on total-tract apparent digestibility of nutrients in mature ewes. DW used in the study was grown on digestate from an anaerobic digester treating dairy wastewater and had 36.9% CP and 16.9% neutral detergent fiber (NDF). Nine ewes were blocked by age and body weight (BW) and randomly assigned to 1 of the 3 treatments. The experiment was a 3×3 Latin square design with 3-wk experimental periods and 2-wk washouts between periods. Fecal, ruminal fluid samples, and BW measurements were collected during the last week of each period and analyzed for nutrient digestibility using indigestible NDF as a marker. Ruminal fluid samples were analyzed for volatile fatty acids (VFA) and ammonia concentrations. Data were analyzed using PROC MIXED in SAS. Dry matter intake was similar among treatments (2.17 ± 0.02 kg/d). Organic matter (OM) and acid detergent fiber digestibility were not affected by DW inclusion ($59.1\% \pm 2.57\%$ and $43.10\% \pm 2.75\%$, respectively). Digestibility of NDF linearly increased ($P = 0.01$) with increasing DW inclusion rate (49.6%, 51.0%, and 56.5% for CON, LDW, and HDW, respectively). Ruminal VFA concentrations were not affected by DW. The molar proportion of propionate was greater ($P = 0.04$) in LDW than CON (19.9% vs. 18.1%, respectively) and tended to be greater ($P = 0.10$) when compared with HDW. Ammonia concentration was not affected by DW. In conclusion, DW inclusion in the diet of mature ewes had no effect on digestibility of ADF and OM, but NDF digestibility increased linearly with DW inclusion level. Ruminal fermentation was generally not affected by DW. Data from this experiment suggest that DW included at up to 10% of dietary DM may have no negative effects on ruminal fermentation and nutrient digestibility in ruminants.

Key Words: duckweed, digestibility, ruminal fermentation

2776 In vitro fermentation evaluation of the effects of ground rosemary leaves on rumen fluid fermentation profiles and redox potential. A. Beckman^{*1}, G. G. Begalli¹, A. F. Lima¹, J. D. de Oliveira^{1,2}, J. Aguado³, T. Fernandes¹, and J. S. Osorio¹, ¹School of Animal Science, Virginia Tech University, Blacksburg, VA, ²Federal University of Grande Dourados, Dourados, MS, Brazil, ³Kemin Industries Inc., Des Moines, IA.

Rosemary leaves (RL) contain several antioxidant compounds, with carnosic acid being the most abundant. This study aimed to investigate the effects of rosemary *Salvia rosmarinus* on rumen fermentation and redox potential using an in vitro fermentation model. Rumen fluid (approximately 400 mL) was collected from a midlactation, cannulated dairy cow (2nd lactation, 103 DIM). The rumen fluid was strained through 4 layers of cheesecloth and mixed with Goering and van Soest medium under CO₂ flux. A total of 100 mL of the buffered rumen solution was dispensed into 250-mL incubation bottles containing either 1 g of total mixed ration (TMR) alone (RL1) or 1 g of TMR and 0.77 mg (RL2), 1.54 mg (RL3), or 3.08 mg (RL4) of ground RL. The bottles were flushed with CO₂, sealed with rubber stoppers, crimped with aluminum caps, and placed in a shaker incubator set at 40 rpm and 39°C. Bottles were incubated for 0, 6, 12, and 24 h. Measurements of pH and redox potential were taken immediately after incubation, and additional samples were frozen and later analyzed for ammonia (NH₃) concentration and volatile fatty acid (VFA) profiles. Data were analyzed using the MIXED procedure in SAS, and orthogonal contrast statements were applied to evaluate linear and quadratic effects of RL supplementation. A linear decrease was observed for isovalerate ($P = 0.05$) as ground RL inclusion rate increased. We observed a treatment $\times$ time interaction ($P < 0.01$) in total VFA. This interaction was mainly driven by a lower ($P < 0.01$) total VFA in RL4 compared with RL1 (88 vs. 124 mmol/L), whereas RL2 and RL3 had intermediate VFA levels (114 and 100 mmol/L, respectively). No significant effects were observed between ground RL inclusion and other VFA, ammonia concentration, pH, and redox potential. The extent of how these effects can be extrapolated to actual rumen fermentation in dairy cows warrants further investigation. Future studies should explore rumen-protection methods to deliver carnosic acid and other antioxidants in rosemary to the lower gastrointestinal tract of ruminants.

Key Words: rosemary, carnosic acid, batch fermentation

2777 Effects of a xylanase and beta-glucanase combination on in vitro rumen fiber digestibility. S. Cronin^{*}, W. Li, T. Parrott, Z. Carlson, F. Mussini, and W. Zeng, Danisco Animal Nutrition & Health (IFF), Wilmington, DE.

Enzymes with fibrolytic activity may help with degradation of animal feed, allowing for greater nutrient release and absorption. In this study, a xylanase and β -glucanase combination product (CX) was tested in vitro for fiber digestibility when applied to different feedstuffs. Six batch culture experiments were conducted in duplicate with different fermentation substrates [alfalfa silage (AS), corn silage (CS), corn stalk (CST), grass hay (GH), dried distiller's grains with solubles (DDGS), and oat hay (OH)]. Enzyme treatments were CX (131.5 U/g of xylanase and 43.8 U/g of β -glucanase) or a no enzyme control (CTR). Feed was weighed (0.50 g DM) into ANKOM bags (F57; ANKOM Technology) that were placed in glass bottles ($n = 4$ /feed per treatment per trial replicate) and incubated for 48 (AS, CS, CST, GH, and OH) or 24 h (DDGS) in buffered rumen fluid. Gas production was measured continuously using the ANKOM RF gas production system. At the end of incubation, pH, DM, NDF, and ADF digestibility were determined. Statistical analysis was performed using the Fit Model procedure in JMP 18 (SAS Institute Inc.), with treatment and feed type as fixed effects

and trial as a random effect. Dry matter digestibility was affected by feed type ($P < 0.01$) and enzyme ($P < 0.01$), with CS displaying the highest digestibility ($72.09\% \pm 1.67\%$) and CST displaying the lowest value ($47.57\% \pm 1.69\%$). When CX was added, DM digestibility was increased to $60.48 \pm 0.70\%$ compared with CTR at $58.45\% \pm 0.72\%$. The interaction of feed type and enzyme was significant for pH, NDF and ADF digestibility, and gas production ($P < 0.01$ for all). Enzyme addition showed increased NDF digestibility in CS, CST, and DDGS (7.63% average increase), increased ADF digestibility in CS and DDGS (9.47% average increase), and increased gas production in CST and OH ($P < 0.01$), but not in other feed types. In summary, in vitro addition of CX increased the fiber digestibilities and gas production of several tested feedstuffs, suggesting greater ruminal fermentation activity.

Key Words: beta-glucanase, xylanase, fiber digestibility

2778 Evaluating the effects of perfluorooctane sulfonate (PFOS) feed binders on the chemical characteristics of simulated abomasal and intestinal fluids. A. Jimenez Lagos^{*1}, M. Chusho Guevara¹, J. Sandro¹, R. Heath¹, M. Mannig¹, T. Wood¹, K. Nishimwe¹, G. Pereira¹, Y. Jiang², and J. Romero¹, ¹University of Maine, Orono, ME, ²University of Wisconsin-Madison, Madison, WI.

This study aimed to evaluate the effects of a collection of perfluorooctane sulfonate (PFOS) feed binders on the chemical characteristics of simulated abomasal and intestinal fluids that were used to sequentially digest pellets resulting from the ruminal digestion of a PFOS-contaminated alfalfa-grass mixture (PFOS: $10 \pm 1.72 \mu\text{g/kg}$; DM basis). Binders included an anion exchange resin (AER), a cationic polymer with quaternary ammonium groups (CPQ), a natural byproduct feed (NBF), a polysaccharide with quaternary ammonium groups (PLQ), a clay binder amended with quaternary ammonium groups (CLQ), and a dioctahedral phyllosilicate clay binder (CLY). The ruminal pellet resulting from the in vitro ruminal incubation [substrate plus binder, and only substrate for untreated substrate (CON)] was rinsed with artificial saliva to remove any unbound PFOS, and then resuspended in a simulated abomasal solution for one h at 39°C , followed by centrifugation. The supernatant was analyzed, and the pellet was rinsed with the artificial abomasal solution and recentrifuged. Next, the pellet was resuspended in a simulated duodenal solution (24 h at 39°C) and subsequently centrifuged to analyze the supernatant and pellets. Data were analyzed using a randomized complete block design ($n = 4$) in SAS v.9.4, with significance declared at $P \leq 0.05$. For pH, the model included the effects of binder type, incubation time, and their interaction, whereas only the effect of binder type was assessed for ammonia-N. The abomasal and intestinal fluid pH levels were not affected by binder type (2.06 ± 0.10 and 7.69 ± 0.03 , respectively). However, after the abomasal incubation, the pH increased from 1.91 to 2.19 ± 0.05 . Conversely, after the intestinal incubation, the pH decreased from 7.75 to 7.64 ± 0.03 . For the abomasal fluid, ammonia-N concentration was lower for CPQ compared with the rest of the binders (3.24 vs. $5.67 \pm 0.29 \text{ mg/dL}$, respectively). For the intestinal fluid, binder type did not affect the ammonia-N concentration [KN1] [JR2] ($5.3 \pm 0.86 \text{ mg/dL}$). The results suggest that most PFOS binders tested have no effects on simulated abomasal and intestinal pH or ammonia-N.

Key Words: abomasal, intestinal, perfluorooctane sulfonate (PFOS)

2779 Postpartum rumination time as a predictor of liver health and calcium status in transition dairy cows. J. A. Ferreyra^{*1,2}, S. Picco², D. Sanchez Otero³, F. Villamil⁴, P. Ricci⁵, D. Colombatto^{1,6}, F. Miccoli^{7,6}, J. M. Cantet⁸, and R. A. Palladino^{1,7}, ¹CONICET, CABA,

Buenos Aires, Argentina, ²UNLP, La Plata, Buenos Aires, Argentina, ³AAMVZ-ICAP-UAEH, Hidalgo, México, ⁴UNNE, Corrientes, Argentina, ⁵UNMdP-INTA, Balcarce, Buenos Aires, Argentina, ⁶FAUBA, CABA, Buenos Aires, Argentina, ⁷FCA-UNLZ, Lomas de Zamora, Buenos Aires, Argentina, ⁸UNER, Concepción del Uruguay, Entre Rios, Argentina.

Transition from pregnancy to lactation is known to be the most challenging period in dairy cows' life. Cow behavior has been proven to be a good indicator of health. Our aim was to correlate rumination (RT), lying, and consumption time against some metabolism indicators. Thirty-eight (38) dairy cows (Fundación Instituto de la Leche, Argentina) were enrolled from -20 to $+21$ DIM to 4 treatments as part of an original study aimed at elucidating the effects of prepartum dietary energy density and seed type (sunflower or linseed) on metabolism and performance of dairy cows during the transition period. Cows were equipped with an automated heat and health monitoring system (Datamars Livestock) to record RT, lying, and consumption time. Blood samples were taken at 4 DIM. Partial correlation analysis was performed using proc glm in SAS, accounting for factors including in the original study (prepartum energy diet, type of seed, time, and their interactions). Significant correlation was declared when $P < 0.05$ and trends when $P < 0.10$ and > 0.05 . Nonsignificant correlations were found between lying time and variables of interest ($P > 0.05$). Consumption time was correlated with aspartate aminotransferase (AST; $r = 0.44$; $P = 0.02$). RT at 4 DIM (RT4) and difference between RT4 and 1 DIM (RT4-1) presented the most interesting results. RT4-1 was positively correlated with milk yield ($r = 0.41$; $P = 0.03$) and negatively with AST ($r = 0.43$; $P = 0.02$). Additionally, RT4 tended to negatively correlate to AST ($r = 0.37$; $P = 0.05$). In addition, RT4 tended to be negatively correlated with alanine aminotransferase (AAT; $r = -0.37$; $P = 0.05$) and positively with cholesterol ($r = 0.33$; $P = 0.08$). Interesting, RT4 correlated positively to Ca levels in blood at 4 DIM ($r = 0.46$; $P = 0.01$) and tended to correlate with Ca difference between 4 and 1 DIM ($r = 0.36$; $P = 0.05$), which means that RT4 may be a good indicator of Ca status at the beginning of lactation. Finally, we calculated the liver health index (LHI) and compared it to the variables under study. No correlation was found between LHI and hepatic enzymes (AST, AAT; $P > 0.05$), but interestingly, LHI correlated positively with Ca at 4 DIM ($r = 0.65$; $P < 0.01$) and with Ca difference between 4 and 1 DIM ($r = 0.42$; $P = 0.02$). Glucose and Mg levels at 4 DIM also correlated positively with LHI ($r = 0.52$ and $r = 0.62$ respectively for glucose and Mg; $P < 0.05$). In conclusion, RT4 postpartum, as well as Ca blood levels during transition, seem to be good indicators of liver health in dairy cows.

Key Words: health monitoring, metabolic adaptation, transition dairy cow

2780 Interaction between ruminal microorganisms and their effects on forage fermentation parameters. D. Cantarelli Rosler^{*}, M. P. Mezzomo, C. A. Pozo, and G. V. Kozloski, Federal University of Santa Maria, Santa Maria, RS, Brazil.

The aim of this study was to evaluate the interactive role of bacteria and fungi on forage degradation in vitro. Dried and ground (1-mm screen) samples of ryegrass (*Lolium multiflorum* Lam.) were weighed (1.5 g) in triplicate in 160-mL flasks and incubated in vitro for 48 h in medium (50 mL of buffer + 50 mL of rumen inoculum) containing or not antimicrobial substances. Penicillin, chloramphenicol, and streptomycin (500 mg/L of each) were used as antibiotics, and cycloheximide (50 mg/L) was used as an antifungal. Three replicate assays were conducted anaerobically in a slow-stir water bath system at 39°C . Treatments were as follows: positive control (BF+, without antimicrobials), fungi (F, with

Table 1 (Abstr. 2780). Ruminal fermentation parameters of *Lolium multiflorum* Lam. incubated with different media¹

Variable	BF+	F	B	BF−	SD	P-value
V (mL)	129 ^a	68 ^b	124 ^a	25 ^c	2.02	<0.0001
Kd (%/h)	4.1 ^{ab}	3.6 ^{bc}	4.3 ^a	3.4 ^c	0.17	0.001
L (h)	−2.9 ^b	−0.8 ^a	−2.9 ^b	−7.0 ^c	0.46	<0.0001

^{a-c}Different superscript letters within rows indicate significant differences.

¹B = antifungal; BF+ = without antimicrobials; F = antibiotic; and BF− = with antimicrobials; Kd = rate of gas production; L = lag time; and V = total gas production. Negative values occurred due to the logistic model used.

antibiotics), bacteria (B, with antifungal), and negative control (BF−, with both antimicrobials). Gas volume was measured at 3, 6, 9, 12, 24, 36, and 48 h, with cumulative gas production data of each essay fitted to a 1-pool logistic model, which generated 3 kinetic parameters: total gas production (mL), rate of gas production (%/h), and lag-time (h). Data were analyzed using the GLM procedure, and the means were compared using the Student *t*-test. The model was $Y_{ij} = \mu + T_i + e_{ij}$, where μ = mean, T = treatment, and e = error. All variables were affected by treatments ($P < 0.05$). Bacteria presented similar values to BF+ for all variables. The BF+ treatment had 53% higher V when compared with fungi, without affecting the gas production rate. In BF−, total gas production was considerably low (Table 1). Low V for BF− might have affected lag time due to the logistic model used. In conclusion, bacteria have the key role in the degradation of ryegrass, with low participation of fungi in this process.

Key Words: fiber degradation, microbiology, rumen

2781 Prediction of rumen pH in dairy cows via exhaled breath metabolomics: Application of machine learning and generalized additive mixed models. M. Z. Islam*, U. Arshad, and M. Niu, *Animal Nutrition, Department of Environmental Systems Science, Institute of Agricultural Sciences, ETH Zürich, Zürich, Switzerland.*

Rumen pH critically influences microbial fermentation, VFA production, and nutrient digestion in dairy cows. Current prediction models predominantly utilize indirect indicators, resulting in moderate predictive accuracy. This pilot study aimed to evaluate the potential of using noninvasive exhaled breath metabolomic (exhalomic) features to predict rumen pH, integrating statistical and machine learning methods. Data were compiled from 2 studies: (1) a switchback design with 4 rumen-cannulated Original Brown Swiss cows fed 6% versus 16% starch in DM across three 9-d periods; and (2) a 3 × 3 Latin square study involving 12 Holstein cows on 3 starch-level diets (12%, 20%, and 28% starch) evaluated over 2 out of three 21-d periods. Exhalome samples were collected at 3-h intervals (8 times/period) using a GreenFeed System and analyzed with secondary electrospray ionization-mass spectrometry. Concurrently, rumen fluid pH was measured using a potentiometer with a glass electrode at 3-h intervals (8 times/period), ranging between 6.31 and 7.01. Random forest and gradient boosting machines prescreened the data set, selecting the top 9 exhalome features characterized by exact mass-to-charge ratios (± 5 ppm) matched against livestock and human metabolome databases. Selected features were then modeled with generalized additive mixed models, incorporating smoothed thin-plate regression splines. Model performance was evaluated via leave-one-out cross-validation, revealing moderate predictive accuracy (RMSPE = 0.312, 4.80% of mean; CCC = 0.406). Key metabolites influencing predictions, identified by Shapley (SHAP) analysis (mean SHAP >0.055), included hexanoate, acetate, lactate, butyrate, and butyronitrile. Hexanoate, butyronitrile, and propionate exhibited nonlinear relation-

ships with rumen pH, whereas acetate (negative) and butyrate (positive correlation) were almost linear. The moderate predictive performance observed was likely due to the limited rumen pH range evaluated. Future research including a broader pH range is recommended to enhance model accuracy, biological interpretability, and applicability in precision dairy management.

Key Words: exhalomics, rumen pH, prediction

2782 Can we assess fermentative and metabolic heat production loss using exhalomics in dairy cows? M. A. Barrientos-Blanco*, U. Arshad, and M. Niu, *ETH Zürich, Zürich, Switzerland.*

Physiological models of energy partitioning assume that fermentation heat loss (FHL) equals urinary energy. Our study aimed to explore a method to quantify the FHL in dairy cows. Twelve multiparous Holstein cows (100 ± 20 DIM; 43.4 ± 6.77 kg/d milk yield) were enrolled in a crossover design (2 wk of adaptation and 1 wk of caloric restriction) and randomly assigned to 2 treatments: control (CON) or negative energy balance (NEB). The CON and NEB diets contained 17.5 vs. 17.2 MJ/kg of DM of gross energy (GE), 38.7% vs. 50.7% NDF, 15.9% vs. 10.5% CP, and 16.5% vs. 7.9% starch, respectively. Milk (M) yield and DMI were monitored daily. The gases (CO₂, O₂, and CH₄) were measured using GreenFeed, along with spot fecal (F) and urine (U) sampling, conducted 8 times during the last 3 d of each period, to represent every 3 h of the day. The GE and N content of diet, F, U, and M were determined through the calorimetric bomb and Dumas method, respectively. Implementing an established method, we differentiated exhalome (Ex, ruminal eructation + breath) and breath (Br) using a threshold of 150 mV CH₄, to get CO₂ and CH₄ emissions, and O₂ consumption for Ex and Br. The adjusted values of gases representing FHL were determined as FHL = Ex − Br g/d. Heat production (HP) for Br (HP_{Br}) and FHL (HP_{FHL}) were calculated as HP = 3.866 × O₂ + 1.2 × CO₂ − 0.518 × CH₄ − 1.431 × urinary N. Energy retention (ER) was determined as ER = diet − U − F − M − CH₄ − HP_{FHL} − HP_{Br}. Data were analyzed using a mixed model with fixed effects of diet and time and random effect of cow. Inducing caloric restriction reduced ($P < 0.01$) the GE intake (368 vs. 545 ± 34.8 MJ/kg of DM) in NEB compared with CON. The caloric restriction led to an increased ($P < 0.01$) partitioning of energy toward U (7.3% vs. 4.6%), F (32.8% vs. 24.9%), CH₄ (6.8% vs. 5.2%), and HP_{Br} (34.1% vs. 27.2%) in NEB compared with CON. Nevertheless, the energy partitioned toward M (22.2% vs. 21.5%) or HP_{FHL} (2.3% vs. 2.0%) did not change across treatments. These findings demonstrate that the major heat energy loss likely originates from metabolic activity rather than fermentative energy production.

Key Words: fermentative energy loss, heat production, exhalome

2783 Association of dry matter intake, 240-hour undegraded neutral detergent fiber, and neutral detergent fiber during the dry period in Holstein cows. L. Garcia* and F. C. Cardoso, *University of Illinois at Urbana Champaign, Urbana, IL.*

Undegraded neutral detergent fiber at 240 h (uNDF₂₄₀) offers an alternative approach to evaluate forage quality and may be helpful in assessing dairy diets, specifically regarding dry matter intake (DMI). However, there is scarce evidence of uNDF₂₄₀ association with DMI for cows during the dry period. Therefore, our objective was to determine the association between DMI prepartum and uNDF₂₄₀ intake based on uNDF₂₄₀ and neutral detergent fiber (NDF) concentrations in dry cow diets. We conducted a pooled statistical analysis of 3 studies, including data from 190 multiparous and 40 primiparous Holstein cows ($n =$

230). Daily DMI was incorporated into the database, with an average DMI of 12.3 ± 4.15 kg/d. Additionally, the average uNDF₂₄₀ was $11.1\% \pm 2.98\%$ of dietary DM, and the average NDF was $37.0\% \pm 4.65\%$ of dietary DM. Both uNDF₂₄₀ and NDF concentrations were divided into tertiles, and cows were categorized as follows: LOW240 (uNDF₂₄₀% $\leq 9.90\%$), MED240 (uNDF₂₄₀% $> 9.90\%$ and $\leq 12.8\%$), HIG240 (uNDF₂₄₀% $> 12.8\%$), LOWNDF (NDF% $\leq 35.1\%$), MEDNDF (NDF% > 35.1 and $\leq 39.8\%$), HIGNDF (NDF% $> 39.8\%$). Statistical analysis was performed using the MIXED procedure in SAS, considering the cow as the experimental unit and the experiment as a random effect. There was no association between DMI and uNDF₂₄₀ concentration nor NDF concentration ($P > 0.20$). There was a tendency for a treatment $\times$ week interaction ($P = 0.09$) in which cows in HIG240 tended to have a greater uNDF₂₄₀ intake at weeks -3, -2, and -1 ($1.80, 1.75$, and 1.53 ± 0.04 kg/d, respectively) than cows in MED240 ($1.54, 1.49$, and 1.29 ± 0.04 kg/d, respectively) and LOW240 ($1.10, 1.08$, and 0.95 ± 0.04 kg/d, respectively). Additionally, cows in HIGNDF had a greater ($P < 0.0001$) uNDF₂₄₀ intake (1.66 ± 0.03 kg/d) than cows in MEDNDF (1.39 ± 0.03 kg/d) and LOWNDF (1.14 ± 0.03 kg/d). In conclusion, DMI prepartum remained consistent despite variations in dietary uNDF₂₄₀ and NDF concentrations, whereas uNDF₂₄₀ intake increased with higher concentrations of dietary uNDF₂₄₀ and NDF.

Key Words: DMI, undegraded neutral detergent fiber at 240 h (uNDF₂₄₀), NDF

2784 Protozoal rumen community structure is affected by dietary branched short-chain fatty acid supplementation and forage level in dairy cows. A. D. Magnuson¹, F. Machado De Sant'Anna¹, K. F. Kalscheur², M. Vedovatto³, N. K. Fukagawa⁴, and J. Kraft^{*1,5}, ¹Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT, ²US Dairy Forage Research Center, USDA-ARS, Madison, WI, ³Louisiana State University, Alexandria, LA, ⁴USDA ARS Beltsville Human Nutrition Research Center, Beltsville, MD, ⁵Department of Medicine, Division of Endocrinology, Metabolism and Diabetes, Burlington, VT, USA.

Protozoa significantly contribute to the total microbial biomass in the rumen and play a role in shaping the microbial community structure. However, the effects of dietary factors on rumen protozoa remain largely underexplored. This study aimed to determine whether branched short-chain fatty acid (BSCFA) supplementation and dietary forage level influence the rumen protozoal community structure. A total of 23 lactating cannulated cows (169 ± 56 d in milk) were randomly assigned to 1 of 4 treatments in a 2×2 factorial design. Treatments included low forage (LF, 50% of the diet on a dry matter basis) or high forage (HF, 65% on a dry matter basis), with or without BSCFA supplementation (2.4 g/kg dry matter of a blend containing *iso*-butyrate, 2-methylbutyrate, and *iso*-valerate in a 2:1:1 ratio). Cows were fed their respective diets for 8 wk, and rumen samples were collected at the end of the study. Following DNA extraction of rumen samples, 18S rRNA long-read sequencing was conducted using Nanopore technology to assess protozoal community composition. Sample richness and α -diversity metrics were analyzed using ANOVA, and β -diversity was evaluated using PERMANOVA. Taxonomic comparisons of abundance were analyzed via 2-way ANOVA and Tukey's HSD. Both forage and BSCFA supplementation individually increased α -diversity (Shannon, Simpson, Simpson's reciprocal index, Berger-Parker's, $P < 0.001$), but no interaction effects were observed. Protozoal β -diversity was unaffected by diet. All classified protozoa belonged to the phylum *Ciliophira*, class *Intramacronucleata*, order *Litostomatea*, and family *Trichostomatia*. HF resulted in a greater abundance of *Buxtonella* spp. ($P = 0.02$), but

decreased *Macropodinium* spp. ($P = 0.03$). BSCFA supplementation reduced abundance of *Polydiniella* spp. ($P = 0.02$) and *Tripulmaria* spp. ($P = 0.02$). In conclusion, both dietary forage level and BSCFA supplementation affected rumen protozoal α -diversity and taxonomic composition.

2785 Rumen protozoal membrane lipids are affected by dietary forage level and branched short-chain fatty acid supplementation. A. D. Magnuson¹, K. F. Kalscheur², M. Vedovatto³, N. K. Fukagawa⁴, and J. Kraft^{*1,5}, ¹Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT, ²US Dairy Forage Research Center, USDA-ARS, Madison, WI, ³Louisiana State University, Alexandria, LA, ⁴USDA ARS Beltsville Human Nutrition Research Center, Beltsville, MD, ⁵Department of Medicine, Division of Endocrinology, Metabolism and Diabetes, Burlington, VT.

Rumen protozoa contribute significantly to the rumen microbial biomass and serve as a potential source of lipids for dairy cows. Although dietary components such as forage type and branched short-chain fatty acids (BSCFA) are known to affect bacterial lipid composition in the rumen, their effects on protozoal lipids remain unexplored. This study aimed to determine if forage level and supplemental BSCFA influence rumen protozoal membrane lipid composition, including fatty acids (FA) and plasmalogens. Twenty-three lactating cannulated cows (169 ± 56 d in milk) were randomly assigned to 1 of 4 dietary treatments in a 2×2 factorial design. The treatments consisted of low forage (LF, 50% of diet dry matter basis) or high forage (HF, 65% of diet dry matter basis), with or without BSCFA supplementation (2.4 g/kg dry matter of a blend containing *iso*-butyrate, 2-methylbutyrate, and *iso*-valerate at a 2:1:1 ratio). Cows were fed their respective diets for 8 wk, after which rumen samples were collected. Protozoa were isolated via centrifugation, and their lipids were extracted and converted into FA methyl esters and dimethyl acetals (DMA), separated using thin-layer chromatography, and analyzed on a Shimadzu GCMS-QP2010. Protozoal community composition was analyzed using 18S rRNA long-read sequencing with Nanopore technology. The results of this analysis are presented in a separate abstract. Treatment effects were assessed with 2-way ANOVA in JMP. HF elevated several FA, including 11:1/12:0, 13:0, 14:0 *iso*, 18:0 *iso*, 18:1 *trans*-6-8, and 18:3 *cis*-9,*cis*-12,*cis*-15 (all $P < 0.05$). BSCFA supplementation reduced 18:1 *trans*-11 ($P < 0.05$). Additionally, HF elevated DMA 18:1 *cis*-12 isomer but reduced DMA 10:0 ($P < 0.05$). The combination of HF and BSCFA supplementation resulted in lower proportions of several individual DMA, including 15:0 *anteiso*, 16:0, 17:0 *iso*, 19:0 *iso*, as well as DMA classes such as total odd, total *iso*, total *anteiso*, total branched-odd, total branched-even, and total branched DMA ($P < 0.05$). These findings suggest that forage level and BSCFA supplementation uniquely influence protozoal lipid synthesis.

Key Words: fatty acid, mass spectrometry, plasmalogen

2786 Effects of drying method and cutting on the methane-emitting potential of legume plants in vitro. C. J. Eifert^{*1}, N. A. Buffington¹, N. Stepanchenko¹, G. Wang², S. Chopra², and A. N. Hristov¹, ¹Department of Animal Science, The Pennsylvania State University, University Park, PA, ²Department of Plant Science, The Pennsylvania State University, University Park, PA.

Several species of legumes are known to produce 3-nitropropionic acid (3-NPA) and have been shown to reduce enteric CH₄ emissions in vitro. The goal of this study was to determine the effect of drying method and cutting on the in vitro CH₄-mitigating potential of field-grown *Securigera*, a 3-NPA-producing legume plant variety (24F1).

A total of 2 incubations were carried out for up to 48 h at 39°C with continuous agitation using an automated gas production system. First cutting (CUT1) 24F1 plants were freeze-dried (FD) or oven-dried at 37 (OD37) or 55°C (OD55). FD CUT1 was compared with a second cutting of FD 24F1 plants harvested from the same field locations 1 mo later (CUT2). Oven-dried alfalfa (AA) hay was the control. All treatments were incubated as fermentation substrate with buffered ruminal inoculum at a 1% (wt/vol) inclusion rate. Total gas production (TGP) was measured continuously, and gas samples were taken at 12, 24, and 48 h for CH₄ and H₂ analysis. Data were analyzed using PROC MIXED in SAS with treatment (24F1 vs. AA), drying method, cutting, and interactions in the model. Incubation was included as a random effect for all comparisons. Compared with AA, CH₄ production was inhibited ($P < 0.001$) 94%, 77%, and 40% by 24F1 at 12, 24, and 48 h, respectively. TGP was 4% and 2% lower ($P = 0.03$) for OD37 than FD and OD55 at 24 h, respectively. No difference in TGP was detected between drying methods at 48 h. Although drying method did not affect CH₄ concentration at any time point, H₂ concentration in TGP for OD37 was 11% and 10% lower ($P = 0.04$) than FD and OD55 at 24 h. No difference in H₂ concentration was detected between drying methods at 48 h. TGP was lower (10%, 7%, and 7%; $P = 0.001$) for CUT2 versus CUT1 at all time points, but CH₄ in TGP increased by 11% ($P = 0.01$) for CUT2 at 24 h. No effect of cutting on CH₄ concentration in TGP was detected at 12 or 48 h. Overall, 24F1 inhibited CH₄ production with decreasing efficacy as the incubation progressed. In this study, CUT2 plants resulted in higher CH₄ concentrations at 24 h, and drying method had no effect on the CH₄-mitigating potential of 24F1.

Key Words: methane, legume forage, ruminal fermentation

2787 Isoacids and condensed tannin combinations: Effects on digestibility and enteric methane emissions in dairy cows using a batch culture system. M. R. A. Redoy^{*1}, M. S. Bari¹, D. Kleinschmit², and M. E. Uddin¹, ¹*Department of Animal Science, University of Connecticut, Storrs, CT*, ²*Zinpro Corporation, Eden Prairie, MN*.

Our objectives were to evaluate (1) 3 isoacids (ISO) and 3 condensed tannin (CT) doses, along with their 9 combinations (ISO+CT), for nutrient digestibility and enteric methane (CH₄) emissions in a dairy diet using a batch culture system (RF system, Ankom Tech, USA), and (2) rank the combinations using breakpoint regression to minimize CH₄ or maximize digestibility, applying a weighted ranking scale (50% CH₄ reduction, 50% digestibility) for a balance. The ISO doses were 0.8 (low), 1.6 (medium), and 3.2 (high) g/kg DM, whereas CT doses were 0.12 (low), 0.24 (medium), and 0.48 (high) g/kg DM. It was a randomized complete block design experiment (6 replications per treatment), where each batch was considered as a block. Rumen fluid was collected via esophageal tubing from 3 Holsteins. Each culture bottle received 20 mL of pooled rumen fluid, 80 mL of buffer, and 1 g of basal diet (16.0% CP and 27.2% NDF) in a filter bag (F57, Ankom Tech, USA) and was incubated for 24 h in a shaking water bath at 39°C. Total gas production (TGP) was calculated from the RF system, and gas samples were analyzed by gas chromatography for CH₄ quantification. Data were analyzed using a mixed-effects model with treatment as fixed effect and block as random effects. Our results showed that combinations (ISO+CT) had similar TGP to CT alone ($P = 0.13$) but were 4% lower than ISO (168 vs. 176 mL; $P = 0.04$). In contrast, ISO+CT reduced CH₄ production by 22% compared with ISO alone (23.7 vs. 19.5 mL; $P < 0.01$) but increased it by 8% relative to CT alone (18.1 vs. 19.5 mL; $P < 0.01$). However, ISO+CT tended to increase DM ($P = 0.07$) and NDF digestibility ($P = 0.08$) by 4% to 5% compared with CT alone, but it was still lower than that of ISO alone ($P < 0.05$). Segmented regression analysis

indicated that the optimal breakpoints for maximizing DM digestibility were at ISO (low) + CT (medium), whereas CH₄ was minimized at ISO (medium) + CT (medium). However, the weighted ranking, which equally prioritized CH₄ reduction and DM digestibility, identified ISO (medium) + CT (medium) as the best combination for overall balance.

Key Words: feed additive, dose optimization, condensed tannin

2788 Feeding proteinate trace minerals and alpha-amylase to lactating cows: Ruminal apparent digestibility of nutrients and ruminal fermentation. R. Chesini¹, N. T. S. Grigoletto¹, C. S. Takiya^{2,1}, G. G. da Silva¹, D. J. C. Vieira¹, A. F. Koontz³, L. F. C. Silva³, and F. P. Rennó^{*1}, ¹*University of São Paulo, Pirassununga, São Paulo, Brazil*, ²*Universidade Tecnológica Federal do Paraná, Pato Branco, Paraná, Brazil*, ³*Alltech, Lexington, KY*.

Trace minerals (TM) fed in inorganic form may impair enzymatic activity and fermentation in the rumen. An experiment was conducted aiming to evaluate ruminal apparent digestibility (RAD) of nutrients and ruminal fermentation of lactating cows fed with proteinate trace minerals (PTM) and α -amylase. Eight ruminal cannulated Holstein cows (29.5 $\pm$ 3.66 kg/d milk yield and 216 $\pm$ 30 DIM) were blocked according to parity and were enrolled into a replicated 4 $\times$ 4 Latin square experiment with a 2 $\times$ 2 factorial treatment arrangement. Experimental periods lasted 21 d, with the first 14 d for diet acclimation. Treatments were as follows: (1) INO, trace minerals in inorganic form (Co, Cu, and Mn as sulfates, Zn oxide, and Se as sodium selenite); (2) INO + AMY, INO treatment and α -amylase fed at 0.5 g/kg diet DM (Amaize, Alltech); (3) PTM, trace minerals in the form of proteinates (Co, Cu, Mn, and Zn – Bioplex, Alltech; and Se-yeast – Sel-Plex, Alltech); or (4) PTM + AMY, PTM treatment and α -amylase fed at 0.5 g/kg diet DM. Dietary TM concentrations followed NASEM guidelines. Ruminal fluid samples were collected on d 15 of each period (every 2 h from the first feeding until 16 h) and VFA concentrations were quantified using gas chromatography. On d 20 (4 h after feeding) and on d 21 (4 h before feeding), the rumen was emptied to determine RAD. Ruminal digesta was weighed and samples were collected for chemical analysis. Data were analyzed using the Mixed procedure of SAS with the fixed effects of Latin square, treatment, period, and the random effect of cow nested in square. Ruminal fermentation data were analyzed using repeated measures. Ruminal concentrations of acetate, propionate, isovalerate, and total VFA were greater ($P \leq 0.02$) in cows fed PTM treatments. Total VFA concentrations were 102, 97.2, 107, and 107 mmol/L for INO, INO+AMY, PTM, and PTM+AMY, respectively. Treatments did not significantly differ in RAP of DM, CP, NDF, or starch. Feeding PTM and Se-yeast increased ruminal concentrations of VFA, and α -amylase had limited effect on ruminal fermentation.

Key Words: enzyme, organic mineral

2789 Effects of gradually increasing dietary starch levels from prepartum to early lactation on nutrient digestibility and microbial composition using continuous culture fermenters. B. J. Schrag^{*}, M. Perdomo, and F. Batistel, *University of Florida, Gainesville, FL, USA*.

Diet management in high-producing dairy cows involves significant shifts in forage-to-concentrate ratios from the prepartum period to early lactation to meet high energy demands. High-starch diets support production during early lactation but may negatively affect the rumen microbiome. We hypothesize that gradual dietary changes during the periparturient period facilitate microbial adaptation and rumen fermentation. Eight continuous culture fermenters were used in a replicated 4

× 4 Latin square design. The 18-d experimental period simulated the transition from prepartum diets (phase 1: d 1–9) with varying starch levels (14%, 17%, 20%, or 23%) to a standard early lactation diet (phase 2: d 10–18) containing 27% starch. Fermenters were fed 27.5 g DM twice daily, and samples were collected from d 7 to 18 to assess nutrient digestibility and microbial composition. Data were analyzed using a mixed model with treatment, day, and their interaction as fixed effects and fermenter as a random effect. In phase 1, starch digestibility increased with starch level, whereas fiber digestibility and ammoniacal N decreased ($P \leq 0.05$). Microbial richness and diversity (Chao1 and Shannon indexes) were highest in the 14% and 23% starch treatments ($P \leq 0.02$). In phase 2, fiber digestibility dropped sharply in fermenters previously receiving lower-starch diets ($P = 0.0$), though differences diminished over time. Starch digestibility and ammoniacal N were unaffected in phase 2 ($P = 0.23$). Diversity remained influenced by treatment ($P \leq 0.05$) but richness was not ($P = 0.11$). Our preliminary results suggest that the magnitude of starch change between prepartum and postpartum diets affects nutrient digestibility due to shifts in microbial community composition.

Key Words: diet composition, microbiome

2790 Previous day passage rates influence consecutive sampling digestibility estimates in dual-flow continuous cultures. B. A. Wenner^{*1} and R. R. White², ¹Feedworks USA, Cincinnati, OH, ²School of Animal Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA.

Dual-flow continuous culture (DFCC) fermenter systems may provide valuable information about the effects of potential feed additives or dietary treatments on fiber digestibility, microbial populations, and gas emissions. However, some of these systems are relatively difficult to operate, with imprecise outflow management leading to natural oscillation of daily passage rates in a bi-daily pattern. Our objective was to evaluate the influence of oscillating passage rate on primary digestibility estimates (NDF, ADF, and VFA) to inform standard procedures on quality control for sampling days during an experiment. The working hypothesis was that days with a low solid passage rate would instigate an apparently high digestibility measurement, followed by increasing flow decreasing apparent digestibility on the next day. Data were collated from 7 previous DFCC studies, all run using the same system, where flow rates (solids: 5% [1.9%–6.7%], total: 7% [3.8%–8.5%]) and daily effluent were recorded and analyzed individually. Data were analyzed using the glmmTMB package of R v4.4.2. Linear, mixed-effect models were derived to estimate measured OM, CP, NDF, and ADF digestibilities with fixed effects for treatment, along with the current (today) and lagged (previous) solid passage rate, total (liquid) passage rate, and daily flow (volume). Study was used as a random effect, and residual error variance was fixed at 1. Digestibility of OM and ADF were significantly associated with both the current and lagged solid passage rate, liquid passage rate, and daily flow ($P < 0.001$ for all variables). Digestibility of NDF was affected by the current and lagged liquid passage rate and daily flow ($P < 0.024$), but not by the current ($P = 0.824$) or lagged ($P = 0.677$) solid passage rate. Apparent digestibility of CP was affected by the current and lagged solid passage rate and daily flow ($P < 0.009$) but not by the current ($P = 0.431$) or lagged ($P = 0.980$) liquid passage rate. These results demonstrate the multi-day effect of oscillating passage rate in some variables of interest within DFCC research and provide the opportunity to educate tolerances for passage rate ranges in experimental quality control.

Key Words: fermenter, rumen, passage rate

2791 Development of a computational fluid dynamics model to optimize fermenter mixing for continuous culture research. J. N. Wenner¹ and B. A. Wenner^{*2}, ¹Department of Mechanical Engineering, University of Wisconsin-Madison, Madison, WI, ²Feedworks USA, Cincinnati, OH.

Dual-flow continuous culture (DFCC) fermenter systems represent a cost-effective research tool to evaluate potential rumen modifiers and mitigate livestock risk. However, the growth in research labs utilizing DFCC leads to methodological divergence from combined protocol drift and research ingenuity. To better evaluate the effect that fermenter configuration may have on mixing dynamics, a computational fluid dynamics (CFD) model was used to simulate previous trials where DFCC configuration details were reported in conjunction with quantified NDF digestibilities. Our working hypothesis was that an orientation of impellers thrusting downward against the bottom of the jar was necessary to maximize mixing. To test this hypothesis, configurations of impeller blades were evaluated at several rotational speeds. Three-dimensional models of configuration geometry were built using computer-aided design. Model dimensions were based on commercially available DFCC equipment and overall configuration dimensions were verified compared with existing physical DFCC in several labs. A CFD model was generated and simulation results were verified for fluid velocities consistent with impeller blade tip velocities, and a steady-state solution was achieved in all compared configurations. For cross-sectional slices distributed throughout the model fluid volume at 50 rpm, the low end of current publications in the field, localized velocities ranged from 98.5 to 3.29×10^5 $\mu\text{m/s}$, primarily driven by proximity to impeller(s) and/or culture surface. For a lower rpm of 25, localized velocities ranged from 43.9 to 9.81×10^4 $\mu\text{m/s}$. Data were analyzed as a mixed model with fixed effect of configuration and/or rotational speed and random effect of cross section. Average fluid velocities with 3 impellers configured to thrust upwards, downwards, and upwards in order from top to bottom in the DFCC equaled 5.54×10^4 $\mu\text{m/s}$ at 50 rpm and were more than double ($P < 0.01$) the velocities at 25 rpm under the same configuration of 2.39×10^4 $\mu\text{m/s}$. Both velocities far exceeded previously estimated protozoal swimming speeds by a magnitude of 100, indicating that known DFCC limitation for protozoal studies may be mitigated by optimizing mixing at lower velocities.

Key Words: continuous culture, fiber digestibility, mixing

2792 Calculating total tract apparent digestibility is not different when adjusting or not adjusting intake for the ort nutrient composition for lactating cow diets. A. L. Kerwin^{*} and T. R. Overton, Department of Animal Science, Cornell University, Ithaca, NY.

Analyzing ort samples to measure total-tract apparent digestibility (TTAD) can be costly. The objective was to compare DM, starch, and ash-corrected NDF (aNDFom) TTAD, using undigested aNDFom after 240 h of incubation as an internal marker, calculated by either using the chemical composition of the TMR and the feces or the chemical composition of the TMR, feces, and orts for lactating Holstein cows. In a method agreement study, multiparous Holstein cows ($n = 28$) between 32 and 71 DIM were enrolled and moved to a freestall pen with Calan gates. Samples were collected on 2 consecutive days at wk 2, 6, and 10 during the 10-wk study and analyzed at a commercial laboratory (Dairy One Cooperative). Bland–Altman plots were generated, and the mean bias between the nutrient digestibility methods was assessed using a paired t test (PROC TTEST; SAS v. 9.4). Pearson correlations between the means and the differences of the 2 methods were assessed using PROC CORR. Lin's concordance correlation coefficient (CCC) was

calculated to assess the agreement between the 2 methods. We observed excellent agreement between the methods for DM (CCC [95% CI] = 0.958 [0.933, 0.974]), starch (0.9998 [0.9997, 0.9999]), and aNDFom (0.9993 [0.9988, 0.9996]); however, there was evidence that the mean difference between methods was different from 0 (mean difference [95% CI]: DM = 0.62 [0.40, 0.85], $P < 0.001$; starch = -0.006 [-0.011, -0.001], $P = 0.03$; aNDFom = -0.056 [-0.115, 0.003], $P = 0.06$). There was a correlation between the bias (the difference between the methods; y-axis) and the magnitude (the mean of the methods; x-axis) for DM ($r = 0.25$; $P = 0.04$; slope = 0.06) and starch ($r = 0.22$; $P = 0.09$; slope = 0.004), but not for aNDFom ($r = -0.07$; $P = 0.61$; slope = -0.002). Overall, there was excellent agreement between methods. The mean bias and correlation between the difference and mean TTAD values observed is minimal and likely not to be biologically significant. To conclude, analyzing the nutrient composition of the diets may not be necessary when calculating TTAD for lactating cow diets.

Key Words: total-tract apparent digestibility, lactating dairy cow, Bland-Altman

2793 Effects of decreasing dietary concentrations of undigested neutral detergent fiber (uNDF) on ruminal kinetics in lactating cows. N. T. Scognamiglio Grigoletto^{*1}, D. J. Cavalli Vieira², R. G. Chesini¹, G. Poletti¹, O. P. Sbaralho¹, M. S. R. Serrasqueiro¹, N. Terassi da Silva¹, C. S. Takiya³, and F. P. Rennó¹, ¹University of São Paulo, Pirassununga, SP, Brazil, ²University of Wisconsin, Madison, WI, ³Universidade Tecnológica Federal do Paraná, Pato Branco, PR, Brazil.

This study aimed to assess the effects of decreasing dietary uNDF concentrations on ruminal kinetics and the relative microbial population in lactating cows. Eight rumen-cannulated Holstein cows (4 primiparous and 4 multiparous, 151.2 ± 61.4 DIM and 33.7 ± 3.0 kg/d milk yield) were blocked by parity and enrolled in a 4×4 Latin square (LS) design experiment, with each period consisting of 21 d and the last 7 d consisting of sampling. Diets were fed as TMR with a 48:52 forage-to-concentrate ratio, and corn silage (CS) was the sole forage source. Dietary uNDF was manipulated using CS harvested at 2 heights above ground level (40 cm [CS40] and 65 cm [CS65]). Treatment sequences with decreasing concentration of uNDF (11.3%, 10.4%, 9.6%, and 8.9% diet DM), comprised 0, 33.3, 66.6, and 100% of CS65, respectively, and the remainder consisted of CS40. Cows were evacuated 4 h postfeeding on D20 and 4 h prefeeding on D21. Total rumen contents were removed, weighed, sampled, and returned. A 10% subsample was filtered to quantify solid and liquid phases for passage rate (K_p), digestion (K_d), and retention time (TMRT). Samples were also processed for bacterial pellet extraction. Data were analyzed by the Mixed procedure in SAS, modeling the fixed effects of treatment, LS, and their interaction. Cow within LS was considered a random effect. Treatment differences were analyzed by linear and quadratic contrasts. Reducing dietary uNDF did not affect K_p . However, the K_d of DM, OM, NDF and CP showed linear increases ($P \leq 0.04$). No effect was observed for K_d of starch. TMRT of uNDF tended to increase linearly (29.7, 30.9, 29.1 and 34.6 h). However, no effect was observed for the TMRT of DM, OM, NDF, CP, and starch ($P \geq 0.20$). Relative microbial populations showed a linear decrease in the population of *Fibrobacter succinogenes* (0.988, 0.911, 0.799 and 0.806). No differences were detected in populations of *Streptococcus bovis*, *Butyrivibrio* group, *B. proteoclasticus*, and *Anaerovibrio lipolytica*. Lower uNDF diets improved the digestion rate without affecting the passage rate, despite smaller *Fibrobacter s.* populations.

Key Words: passage rate, pool ruminal, microbial population

2794 Decreased concentrations of undigested neutral detergent fiber (uNDF) in diets of dairy cows affect omasal nutrient and microbial protein flow. N. T. Scognamiglio Grigoletto^{*1}, D. J. Cavalli Vieira², R. G. Chesini¹, N. P. Martins¹, O. P. Sbaralho¹, M. S. R. Serrasqueiro¹, N. Terassi da Silva¹, C. S. Takiya³, and F. P. Rennó¹, ¹University of São Paulo, Pirassununga, SP, Brazil, ²University of Wisconsin, Madison, WI, ³Universidade Tecnológica Federal do Paraná, Pato Branco, PR, Brazil.

This study aimed to evaluate the effect of decreasing concentrations of uNDF in the diet of lactating cows on omasal digesta and microbial protein flow. Eight rumen-cannulated Holstein cows (4 primiparous and 4 multiparous, 151.2 ± 61.4 DIM, and 33.7 ± 3.0 kg/d milk yield) were blocked by parity, milk yield, and DIM. Subsequently, the cows were randomly assigned to treatments in a replicated 4×4 Latin square (LS) design, balanced for residual effects. Each experimental period lasted for 21 d, with the last 7 d for data sampling. Cows within blocks were allocated to treatment sequences with decreasing concentrations of uNDF (11.3%, 10.4%, 9.6%, and 8.9% of diet DM). Dietary uNDF concentrations were manipulated using corn silage (CS) made from plants harvested at 2 heights above ground level (40 cm [CS40] and 65 cm [CS65]). Diets were offered as TMR with a 48:52 forage-to-concentrate ratio. CS was the sole forage source. For diets containing 11.3%, 10.4%, 9.6%, and 8.9% uNDF, the forage component consisted of 0%, 33.3%, 66.6%, and 100% CS65, respectively, with the remainder composed of CS40. Microbial protein synthesis was evaluated using the omasal sampling technique along with the double marker system of digesta flow and purines as a microbial marker. Data were analyzed by the Mixed procedure in SAS, modeling the fixed effects of treatment, LS, and their interaction. Cow within LS was considered a random effect. Treatment differences were analyzed by linear and quadratic contrasts. A decreasing dietary uNDF concentration showed a linear tendency to increase PAB flow (particle-associated bacteria; $P = 0.06$), whereas no differences FAB (fluid-associated bacteria) were detected in FAB flow. The omasal flow on DM, OM, NDF, CP and starch, NANMN flow (nonammonia nonmicrobial nitrogen; g/d) and microbial NAN flow (microbial nonammonia nitrogen; 313, 294, 239, and 236 g/d), and that associated with the LP (large particle phase; 373, 369, 347, and 368 g/d) and SP (small particle phase; 151, 175, 161, and 170 g/d) fractions of the omasal digesta, did not show any differences between treatments ($P \geq 0.30$). However, the lowest concentrations of uNDF exhibited a linear increase ($P \leq 0.05$) with respect to nitrogen degradable in rumen (g/d), and g/kg of NAN/kg of OMTDR efficiency decreased linearly (32.7, 28.9, 22.7, and 25.9; $P = 0.039$). Therefore, lowering dietary uNDF concentrations appears to enhance nitrogen degradable in the rumen (g/d) and reduce the efficiency of NAN per kilogram of OMTDR.

Key Words: carbohydrate, fiber indigestibility, omasal flow

2795 Effects of peptide, urea, and branched-chain volatile fatty acid (BCVFA) dosing on peptide and ammonia concentrations in continuous cultures with limiting dietary RDP. A. R. Sanders^{*1}, A. F. White⁴, B. A. Wenner³, D. H. Kleinschmit², M. T. Socha², and J. L. Firkins¹, ¹Department of Animal Sciences, The Ohio State University, Columbus, OH, ²Zinpro Corporation, Eden Prairie, MN, ³Feedworks USA, Cincinnati, OH, ⁴Agri-King, Fulton, IL.

Previous researchers have investigated how peptides and NH_3 -N stimulated microbial growth in pure or batch cultures; however, supplemental BCVFA with peptides has rarely been studied. Our objective was to investigate the effects on peptide and NH_3 concentrations in dual-flow continuous cultures fed limited dietary RDP and dosed with peptides (PEP), urea, and/or BCVFA. We hypothesized that dosing PEP and urea

would inhibit peptidolysis, increasing peptide accumulation, particularly when combined with BCVFA. Six treatments were included in a 6 × 6 Latin square: negative (NCON, 8.5% RDP) and positive (PCON; 10.0% RDP) controls formulated using NASEM (2021). In low RDP diets, PEP (2 g/d) were included in 4 treatments arranged factorially with low (0.15 g/L) and high (0.4 g/L) urea buffers and without and with BCVFA (2.15 mmol/d of each of the 3 BCVFA). Periods had 7 d of adaptation and 4 d of sampling. Liquid and solid dilution rates were 10 and 5%/h. Samples were collected bi-hourly 0.5 to 8.5 h postfeeding, passed through a 0.45-μm filter, and stored at -80°C. An aliquot was filtered through a 10-kDa micropore filter to represent peptides, which were quantified by Kjeldahl after subtracting NH₃-N analyzed by colorimetric assay. The statistical model included the fixed effect of treatment and random effects of period and fermenter. Contrasts were for NCON versus 4 treatments with PEP, PCON versus 4 PEP treatments, and the main effects and interaction of urea with dosed BCVFA within the 4 PEP treatments. Dosing PEP and urea increased ($P < 0.01$) peptide-N and NH₃-N concentrations compared with CON. However, treatment interacted with time ($P < 0.01$); in PEP treatments, BCVFA interacted with urea ($P \leq 0.07$) at 4.5 and 6.5 h such that urea plus BCVFA increased peptide-N concentrations by 49% and 61% compared with PEP without BCVFA and with low urea. Supplementation of PEP and urea, particularly when BCVFA were dosed, increased peptide accumulation through feedback inhibition, and timing coincided with greatest availability of carbohydrate from feeding for protein synthesis.

Key Words: peptide, isoacid, continuous culture

2796 Gossypol reduces methane production in vitro. T. Brand¹, R. Khurana¹, J. Hummel², and M. Hünnerberg², ¹Mootral GmbH, Berlin, Germany, ²University of Göttingen, Göttingen, Germany.

This study investigated the effects of gossypol on in vitro methane (CH₄) production and fermentation kinetics. Two hundred milligrams of a high-forage diet (68.5% forage) were incubated together with 0%, 0.20%, 0.40%, 0.80%, and 1.60% gossypol (all DM basis) extracted from cottonseeds (≥95% purity). Incubations were carried out in gas-tight glass syringes (modified Hohenheim gas test) using a mixture of buffer (20 mL) and rumen fluid (10 mL). The rumen fluid was obtained from 2 nonlactating, rumen-cannulated Holstein-Friesian cows maintained on a hay-based diet. For each treatment, triplicate syringes were incubated (39°C) in 2 separate runs ($n = 6$). The 2 runs were carried out under identical conditions on different days. Triplicate syringes containing only buffer solution and rumen fluid were included as blank controls in both runs. All syringes were incubated for 24 h. Aside from CH₄, H₂, and total gas production, concentration of NH₄-N, volatile fatty acids, and apparent substrate disappearance (DM and OM basis) were assessed. Data were analyzed using the PROC MIXED procedure in SAS. The model included the fixed effect of treatment (gossypol dosage). Incubation run was considered a random effect. Orthogonal polynomial contrasts were used to examine the linear and quadratic effects of increasing doses of gossypol. Supplementing 0.40%, 0.80%, and 1.60% gossypol led to a reduction in CH₄ per gram of DM and OM disappearance, compared with the control and 0.20% gossypol (all $P < 0.05$). To some extent, the reduction was driven by a linear increase in propionate in response to adding gossypol ($P < 0.001$). However, supplementing gossypol linearly reduced total gas production (mL/g DM and mL/g DM disappearance; both $P < 0.001$), as well as DM and OM disappearance ($P < 0.001$), which potentially could have negative implications in vivo. Therefore, further studies verifying the efficacy of gossypol as CH₄ inhibitor should be conducted.

Key Words: in vitro fermentation, gossypol, methane

2797 Bacilli as a tool to support in vitro nutrient degradability of forage-based feedstuffs and total mixed rations. A. C. Storm¹, O. C. M. Queiroz¹, R. Gresse¹, M. Brioso², F. Clemente², I. S. Yu³, and B. I. Cappellozza¹, ¹Novonesis, Kongens Lyngby, ²Novonesis, Argentina, ³Novonesis, Malaysia.

Optimal ruminal nutrient degradation and utilization have a direct effect on the amount of energy available for growth and productive responses in growing and lactating cows, respectively. *Bacillus* spp. have been recognized as outstanding enzyme producers, which could provide additional benefits from the well-reported “probiotic effects” in livestock. Therefore, we hypothesized that adding a combination of *Bacillus licheniformis* and *Bacillus subtilis* would improve the fiber degradability of forage-based substrates and total mixed rations (TMR). Hence, our objective was to evaluate, through a meta-regression analysis, how in vitro incubation of *B. licheniformis* 809 and *B. subtilis* 810 would affect the neutral detergent fiber (NDF) degradability of forage-based substrates ($n = 50$) and TMR ($n = 19$). Forage-based substrates were collected from Argentina, Australia, Brazil, China, and South Africa, whereas TMR were collected from commercial dairy farms located in Argentina, Brazil, the United States, and Vietnam. Treatments included in all analyses consisted of a nonsupplemented control (CON) or the incubation of a *Bacillus*-based direct-fed microbial consisting of *B. licheniformis* 809 and *B. subtilis* 810 (BAC; Bovacillus, Novonesis, Lyngby, Denmark). All data were analyzed using the PROC REG in SAS (version 9.4; SAS Inst., Cary, NC) and tested for quadratic effects and then linear responses. For the forage-based substrates, a quadratic effect was observed in the model ($P < 0.0001$; Figure 1-A). As an example, BAC incubation resulted in 7.8%, 11.8%, and 27.7% greater in vitro NDF degradation versus CON when feedstuffs presented 50%, 60%, or 70% of NDF (dry matter basis), respectively. For the TMR, linear effects ($P < 0.0001$; Figure 1-B) were observed on in vitro NDF degradability. Improvements were in the order of 9.0%, 7.9%, and 7.3% when the TMR contained 27%, 33%, and 39% of NDF (dry matter basis), respectively. In summary, these results suggest that *Bacillus*-based direct-fed microbials may be a feasible nutritional tool to support the degradation of nutrients, more specifically fiber, in the rumen via their enzymatic ability.

Key Words: *Bacillus*-based direct-fed microbial, fiber, forage

2798 Multiomics analysis reveals that the rumen microbiome and its metabolome jointly contribute to individual dairy cow NDF utilization. B. Y. Ma¹, J. X. Xiao², Y. M. Xu³, T. Y. Chen¹, D. Gao¹, S. Liu¹, G. B. Hou¹, S. D. Zou¹, and Z. J. Cao¹, ¹State Key Laboratory of Animal Nutrition and Feeding, College of Animal Science and Technology, China Agricultural University, Beijing, China, ²Institute of Animal Nutrition, Sichuan Agricultural University, Sichuan, China, ³College of Animal Science, Xinjiang Agricultural University, Xinjiang, China.

Increasing neutral detergent fiber (NDF) utilization is critical to improving dairy cow profitability, and NDF digestion efficiency is influenced by rumen microbial activity; this study aims to unravel the microbial-driven mechanisms underlying NDF catabolism and its effects on nutrient utilization and economic benefits. To obtain individual cows with high/low NDF digestibility differences in the natural state, we collected fecal and dietary samples from 131 peak-lactation cows for 3 consecutive days at 4 h after the morning feeding and determined the apparent digestibility of nutrients in each cow via the hydrochloric acid insoluble ash method. Then, 20 cows with the highest and the lowest NDF digestibility (high/low 10: 63.61% ± 2.15% vs. 42.97% ± 1.11% digestibility, $P = 0.01$) were selected. Next, rumen contents were col-

lected and characterized for volatile fatty acids (VFA), and microbial composition and metabolism differences were determined by metagenomics and metabolomics. Statistical analyses included mixed models (GLIMMIX), Kruskal-Wallis tests, LefSe, and Spearman correlations (FDR-corrected $P < 0.05$). The findings suggested that the high-NDF group was significantly enriched for *Spirochaetota* and *Fibrobacterota* at the phylum level, and fewer *Methanobrevibacter* spp. at the species level; meanwhile, alanine aspartate and glutamate metabolism and the valine leucine and isoleucine degradation pathways were significantly enriched in the High-NDF group. Metabolomics analysis revealed elevated amino acid (Ala-Asp-Ile), lipid ([8]-gingerol), and cofactor/vitamin metabolism metabolites in high-NDF group. The contribution of VFA, microbial composition, and metabolite differences in the utilization of NDF was estimated as 54.29%, 46.01%, and 2.70% respectively, by the linear mixed effect model. Rumen microbiota-VFA-metabolite interactions drive NDF digestibility differences. Enriching fibrolytic taxa and suppressing methanogens could enhance feed efficiency. Findings support microbiota-targeted strategies (dietary interventions or genetic selection) to reduce costs and environmental effects.

Key Words: dairy cattle, rumen metagenomic, rumen metabolome

2799 Increasing levels of sodium fluoride in lactating dairy diets in a dual-flow continuous culture system. A. C. S. Vicente^{*1}, G. K. Salas-Solis¹, M. L. Johnson¹, C. A. Hammond¹, A. S. R. Fonseca², A. C. da Costa², and A. Faciola¹, ¹University of Florida, Gainesville, FL, ²Universidade do Mato Grosso, Cuiaba, Mato Grosso, Brazil.

Sodium fluoride (NaF) may affect ruminal fermentation, but the threshold levels at which it exerts an effect remain unknown. Commercially available dietary buffers contain different levels of NaF. The objective of this study was to evaluate the effects of increasing levels of NaF in lactating dairy diets on ruminal fermentation in a dual-flow continuous culture system. Eight fermenters were used in a 4×4 replicated Latin square with 4 periods of 10 d each, evaluating the following treatments: NaF0, S-Carb, at 1% of the diet, without NaF in the diet; NaF1, Trona Carb, at 1% of the diet, totaling 0.0101% NaF in the diet; NaF2, Trona Carb, at 1% of the diet + NaF totaling 0.0202% NaF in the diet; and NaF3, Trona Carb, at 1% of the diet + NaF totaling 0.0303% NaF in the diet. The basal diet was formulated to have similar nutritional composition, with 17% CP; 30.4% NDF; 41% NFC (DM basis) and they were provided twice daily, totaling 106 g/d. After 7 d of adaptation, samples were collected for 3 d of each period for analyses of ruminal fermentation at 0, 1, 2, 4, 6, and 8 h after the morning feeding. Statistical analysis was performed with the mixed procedure in SAS, data using time points were analyzed using repeated measures, and orthogonal polynomial contrasts were used to depict linear and quadratic effects. There were no treatment effects for pH ($P = 0.89$) and ammonia concentration ($P = 0.30$) throughout the day. The LSM ($\pm$ SEM) for pH were 6.23 (± 0.121), 6.21 (± 0.121), 6.24 (± 0.121), and 6.25 (± 0.121) for treatments NaF0, NaF1, NaF2, and NaF3, respectively. The LSM ($\pm$ SEM) for ammonia were 15.16 (± 0.967), 14.87 (± 0.967), 16.12 (± 0.967), and 15.21 (± 0.967) mg/dL, respectively. These results indicate that the tested NaF levels do not affect ruminal fermentation of lactating diets in vitro. Further analyses are needed to assess the effects of these levels on other ruminal fermentation variables, nutrient utilization, and ruminal microbial protein growth.

Key Words: buffer, fluorine, NaF, pH, sodium

2800 Bacterial communities improve rumen in vitro fiber degradation. J. Y. Zhang¹, R. Ma², J. G. Gu², L. Ma¹, and D. P. Bu^{*1},

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This experiment was conducted to evaluate the effect of bacterial communities on in vitro fiber degradation. Four groups of rumen microbial communities were synthesized artificially through culturomics (SynCom 1, SynCom 2, SynCom 3, and SynCom 4) and were arranged into 4 treatment groups accordingly after 14 h of culture. One milliliter of sterile distilled water was the control. Approximately 0.75 g of ground TMR substrate was added into preweighed filter bags. Prewarmed medium (45 mL) and the appropriate strained rumen fluid (15 mL) were mixed into each bottle purged with CO₂ to remove air. Then, this was mixed with the 4 treatment groups and the control and incubated at 39°C for 48 h, with 4 replicates in each group. After fermentation, the substrate was collected and analyzed for dry matter (DM) and neutral detergent fiber (NDF). Fermentation fluids were collected to detect VFA, pH, and gas production. Data were averaged per group and analyzed using ANOVA in R. Statistical significance was declared at $P < 0.05$. SynCom 2 increased pH (6.44 ± 0.02) significantly compared with the control (6.36 ± 0.05) and SynCom 3 (6.36 ± 0.03). The concentration of propionate in SynCom 3 (29.07 ± 0.72) and SynCom 4 (30.28 ± 2.64) was significantly higher than that in the control group (26.15 ± 2.12). The lower acetate concentration coupled with higher propionate production resulted in lower A:P ratios in SynCom 4 (1.74 ± 0.16) than in the control group (1.96 ± 0.05 , $P < 0.05$). However, total gas production was consistently produced at higher values in SynCom 3 (128 ± 9) than the control group (108 ± 4 , $P < 0.05$) which indicated that SynCom 3 improved the activity of rumen microorganisms. Additionally, SynCom 3 increased NDF digestibility (59.75 ± 6.20) by 10.13% compared with the control group (49.62 ± 4.55). Finally, the only difference between SynCom 3 and SynCom 4 was the lower concentration of *P. acetatigenes* 6076 of the bacterial communities in the 2 groups. These findings suggest that specific bacterial communities could improve fiber degradation and the concentration of special strains may further improve feed NDFD in in vitro fermentation systems.

Key Words: dairy cow, fiber degradation, rumen fermentation

2801 Flavonoid-rich extract of *Calotropis gigantea* and quercetin reduce rumen protozoa and ammonia nitrogen production in vitro. A. Abrahaley¹, L. Ma¹, J. C. Xu^{1,2}, and D. P. Bu^{*1}, ¹State Key Laboratory of Animal Nutrition and Feeding, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, ²World Agroforestry Center, Kunming, China, ³Honghe Center for Mountain Futures, Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan, China.

The activity of rumen protozoa, particularly *Entodinium*, is related to inefficiency of nitrogen utilization in ruminants. Flavonoids, known for their potential to reduce rumen protozoal populations, ammonia nitrogen (NH₃-N), and methane (CH₄) production, are of interest for improving nitrogen efficiency in ruminants. This study investigated the effects of *Calotropis gigantea* flavonoid-rich extract (CGFE), quercetin, and myricetin on rumen protozoa, microbial crude protein (MCP), NH₃-N, and CH₄ concentration using an in vitro fermentation system. The ethanolic extract of *Calotropis gigantea* contained 4,855.83 µg QE/g of total flavonoids, including 2,367.25 µg of quercetin and 585.45 µg of myricetin per gram of CGFE. Rumen inoculum from dairy cows was incubated for 24 h with 4 treatment groups: control (no addition), quercetin (8 mg/mL), myricetin (8 mg/mL), and CGFE (8 mg/mL). Three replicates per sample were analyzed in 3 independent

runs. Protozoa were counted in a Sedgewick Rafter chamber at 100× magnification following formalin fixation and brilliant green staining, with morphological identification. Statistical analyses were performed using R software (v4.3.2), with significance set at $P < 0.05$. One-way ANOVA was used to compare treatment means, followed by Tukey's test for pairwise comparisons. Quercetin and CGFE significantly reduced NH₃-N by 31.8% (14.6 mg/dL) and 16.4% (17.9 mg/dL), respectively ($P < 0.001$), whereas myricetin had no effect. Quercetin also increased MCP production by 103.7% (0.55 mg/mL) ($P < 0.001$), whereas myricetin and CGFE had no effect. Quercetin and CGFE reduced total protozoa by 5.8% and 3.3%, respectively ($P < 0.001$), with no effect from myricetin. *Entodinium* counts decreased by 5.7% (5.15 log₁₀ cells/mL) with quercetin and 3.3% (5.28 log₁₀ cells/mL) with CGFE ($P < 0.001$), with no change for myricetin. CGFE did not affect methane production ($P > 0.05$), whereas quercetin and myricetin increased methane by 7.5% and 7.9%, respectively ($P < 0.001$). These findings suggest that quercetin in CGFE has the potential to be an effective additive in reducing rumen protozoa, lowering ammonia production, and improving nitrogen utilization in ruminants.

Key Words: ammonia production, *Calotropis gigantea* flavonoid, rumen protozoa

2802 Effects of nitrate with lactate on nitrate reduction, rumen fermentation, and methane production in an in vitro batch culture. B. Bae^{*1}, L. Pope¹, K. Park^{1,2}, and C. Lee^{1,2}, ¹Department of Animal Sciences, The Ohio State University, Wooster, OH, ²Interdisciplinary Nutrition Program, Department of Animal Sciences, The Ohio State University, Wooster, OH.

Nitrate is a promising methane-mitigating additive but could be toxic when fed to cows depending on the dosage level. Because nitrate and nitrite reduction can be facilitated by lactate-utilizing bacteria, the objective of the experiment was to determine the combined effect of nitrate and lactate. An in vitro batch culture was conducted with 4 treatments: a basal diet (CON) and the basal diet with nitrate (2.2% of DM, Nit), lactate (2.2% of DM, Lact), or the combination of nitrate and lactate (Nit-Lact). Strained rumen fluids from 2 cannulated cows were mixed with McDougall's buffer (1:3) for the in vitro batch culture. The incubation was repeated twice. Data were analyzed using PROC MIXED in SAS with a random effect of incubation and a fixed effect of treatment. The main effects of nitrate and lactate and their interaction were determined. A trend of interaction between nitrate and lactate for DM digestibility (24 h; $P = 0.06$) occurred because DM digestibility tended to decrease for Nit compared with CON (42.2% vs. 45.3%) but did not decrease for Nit-Lact (46.6%). Nitrate decreased ($P < 0.05$) methane and total gas production over 24 h, but no interaction between nitrate and lactate was observed. Although total VFA production was not affected, nitrate increased ($P < 0.01$) acetate and decreased ($P < 0.01$) propionate, butyrate, and valerate as proportions of the total VFA at 24 h of incubation. The concentration of NO₃-N for Nit and Nit-Lact decreased slowly from 0 to 3 h of incubation (77 to 45 mg/L) and became minimal at 6 h. The concentration of NO₂-N for Nit and Nit-Lact was detected but minimal during 24 h of incubation. The concentration of NO₃-N was lower, but NO₂-N was greater for Nit-Lact ($P < 0.05$) compared with Nit at 3 h of incubation. In conclusion, nitrate was effective in decreasing methane. Although nitrate alone tended to decrease DM digestibility compared with CON, no decrease for Nit-Lact suggests that the combination has potential to lower the risk of nitrate toxicity. Lowering the risk of nitrate toxicity for Nit-Lact is supported by the rapid reduction of nitrate compared with Nit.

Key Words: digestibility, nitrite, nitrate toxicity

2803 Effects of *Aspergillus oryzae* and *Aspergillus niger* on the in vitro nutrient degradability of dairy rations. L. V. Vieira^{1,2}, M. R. Pupo^{*1}, M. N. Corrêa², and L. F. Ferraretto¹, ¹University of Wisconsin-Madison, Madison, WI, ²Federal University of Pelotas, Pelotas, Rio Grande do Sul, Brazil.

The mycelium of *Aspergillus* spp. can facilitate enzyme release and increase bacterial concentration in the rumen. These fungi can produce enzymes such as cellulase, pectinase, α-amylase, protease, and ligninase and hence contribute to ruminal digestion. For example, *Aspergillus oryzae* in combination with *Aspergillus niger* have shown greater enzymatic production. However, potential synergistic effects of their combination on nutrient degradability are not well defined. The objective of this study was to evaluate the effects of a coculture of *A. oryzae* and *A. niger* (AOAN) on the in vitro degradability of dry matter (dDM), organic matter (dOM), and neutral detergent fiber (dNDF) of dairy rations in a 2 × 3 factorial arrangement of treatments. Treatments were a corn silage-based diet (40% DM), partial replacement of corn silage with soybean hulls (10% DM), and partial replacement of corn silage with wheat straw (10% DM), as well as inoculation without (CON) or with 0.02% DM of AOAN (American Biosystems, Roanoke, VA). Duplicated 0.5-g samples were weighed into F57 bags and incubated within 250-mL wide-neck glass bottles containing rumen fluid, buffer media, plus micro- and macrominerals. Incubation time points were 12, 30, 48, and 120 h in 3 independent runs. Data were analyzed separately for each diet as a completely randomized design using a mixed model including AOAN as a fixed effect and each independent run as a random effect. Organic matter digestibility (97.0% vs. 96.8% DM; SEM = 0.08) was greater ($P = 0.04$) and dDM (65.2% vs. 64.3% DM; SEM = 0.79) tended to be greater ($P = 0.07$) for wheat straw diets inoculated with AOAN compared with CON at 30 h, respectively. No effects on the in vitro nutrient degradability were observed for soybean hull diets ($P > 0.10$). Digestibility of OM (97.6% vs. 97.5% DM; SEM = 0.05) tended ($P = 0.06$) to be greater at 48 h for CON compared with AOAN in corn silage-based diets. Similarly, greater ($P = 0.03$) dOM (97.7% vs. 97.5% DM; SEM = 0.06) was observed for CON compared with AOAN at 120 h in corn silage-based diets. These results suggest that inoculating AOAN in diets with partial replacement of corn silage with wheat straw might improve nutrient degradability. However, further investigation in vivo is warranted.

Key Words: corn silage, soybean hull, wheat straw

2804 Effects of red-brown seaweed mixture on dry matter, organic matter, nitrogen digestibility, and volatile fatty acid production on in vitro rumen fermentation cultures. M. P. Marin¹, I. Beltran², P. Morales¹, J. Munoz³, M. Avila³, and P. Melendez^{*4}, ¹School of Veterinary Medicine, Faculty of RRNN & Veterinary Medicine, University Santo Tomás, Chile, ²Agriculture & Livestock Research Institute, INIA-Remehue, Chile, ³Aquaculture & Fish Centre (CAPIA), University Santo Tomás, Chile, ⁴Jockey Club College of Veterinary Medicine & Life Sciences, City University of Hong Kong, Hong Kong.

This study aims to report the effect of brown algae found on the northern coast of Peru in the southern Patagonia region of Chile and Argentina and red algae found in the northern region of Chile on volatile fatty acid (VFA) production and dry matter (DM), organic matter (OM), and nitrogen (N) digestibility in vitro cultures. The combination of red/brown algae at inclusion proportions of 100/0, 75/25, and 50/50, respectively, was tested at a concentration of 2% and 5% of DM with a diet of 70% pasture (*Lolium perenne*) and commercial concentrate. Each treatment was carried out in triplicate, and digestibility was measured at 24 h of incubation. Statistical analysis comprised a 2-way ANOVA (treatment

Table 1 (Abstr. 2804).

Item	Proportion red/brown seaweed				SEM	Dose, %		SEM	P-value	
	Control	Red 100	Red 75	Red 50		2	5		Algae	Dose
Digestibility 24 h										
DM, %	88.3 ^a	85.3 ^{ab}	84.7 ^b	85.4 ^{ab}	1.07	86.4	85.5	0.89	0.04	0.33
OM, %	86.7 ^a	82.7 ^{ab}	82.0 ^b	82.9 ^{ab}	1.43	84.2	83.0	1.18	0.04	0.30
N, %	70.0 ^a	62.8 ^{ab}	62.1 ^b	64.5 ^{ab}	3.13	65.5	64.1	2.87	0.03	0.44
VFA 24 h										
Total, mmol/L	93.3 ^a	76.7 ^b	75.6 ^b	78.8 ^b	1.08	81.2	81.1	0.87	<0.01	0.91
Acetate, %	53.2 ^a	48.4 ^b	48.8 ^b	48.6 ^b	0.90	50.2	49.3	0.71	<0.01	0.28
Butyrate, %	13.6 ^b	18.5 ^a	18.0 ^a	17.9 ^a	2.07	16.8	17.2	2.06	<0.01	0.20
Propionate, %	23.1 ^b	31.1 ^a	31.3 ^a	31.5 ^a	0.53	29.0	29.5	0.50	<0.01	0.10
Others, %	2.55	2.65	2.50	2.60	0.52	2.6	2.6	0.51	0.86	0.91

^{a,b}Different superscript letters within rows indicate significant differences.

and dose) and their interaction. LSM were reported at 12 and 24 h after incubation. No negative effect of the inclusion dose or red/brown algae ratio on the in vitro digestibility of DM, OM, or N was observed. Only the inclusion ratio of 75/25 red/brown algae showed a slightly lower digestibility of DM, OM, and N. At 12 h of incubation, total VFA production was not affected by treatment; however, the proportion of propionate was significantly higher in all algae groups than in the control group ($P < 0.01$). When analyzing the results at 24 h of incubation, it was observed that all treatments had a total VFA production lower than the control, which indicates a lower fermentation dynamic. In relative terms, this reduction was 17.8%, 19%, and 15.5% for 100% red algae and the 75/25 and 50/50 red/brown algae ratios, respectively (Table 1). There was no interaction between treatment and dose for any outcome. It is concluded that the combination of red/brown algae slightly reduces the digestibility of DM, OM, and N and the production of VFA at 24 h, with a higher proportion of propionate than in the control group.

Key Words: methane, seaweed, rumen fermentation

2805 In vitro simulation of rumen drinking and effects of using yeast probiotic Actisaf Sc47 on this phenomenon in terms of degradability. N. Salah*, P. Ly, B. Gestes, M. Briche, H. Legendre, E. Paiva, J. Duclos, and M. Engelen, *Phileo by Lesaffre*.

Ruminal drinking (RD) is a recurring phenomenon in preruminant calves and is observed where milk is directed to the rumen instead of the abomasum. Ruminal drinking causes bloating, maldigestion, acidosis and consequently decreased growth performance. The objective of this study was to simulate RD and to evaluate the effect of using a yeast probiotic (Actisaf Sc47, *Saccharomyces cerevisiae* CNCM I-4407). Ankom and Daisy incubator systems were used. Eight calves aged 112 d, of which 4 in control group did not receive Actisaf Sc47 and 4 in experimental group did receive Actisaf Sc47 at 50×10^9 cfu in top dressing, were used as rumen fluid donors. To determine the quantity of milk that could potentially cause RD in calves, estimations were made based on the rumen volume at 112 d (20,900 mL), the quantity of milk that can cause RD (+25% of distributed milk), the critical ruminal pH (≤ 5.5), and the quantity of milk distributed during our trial at 112 d (9,600 mL). It was therefore estimated that 2,400 mL of milk could induce RD in calves, which would translate into 1.6 mL of milk mixed with 14 mL of rumen fluid to simulate RD in the Ankom system and 55.11 mL of milk mixed with 400 mL of rumen fluid to calculate degradability with Daisy incubator system. To validate our approach, we conducted an Ankom trial during which we compared the evolution of pH of rumen fluid sampled from control and experimental calves. A

t-test was performed to compare the difference between the means of the 2 groups. The level of statistical significance was set at $P < 0.05$. A tendency for significance was declared at $0.05 \leq P < 0.10$. For each group, 3 treatments were assessed in triplicate Ankom bottles: control without any milk addition, 30% (1.92 mL) of milk addition, and 40% (2.57 mL) of milk addition. Critical ruminal pH was reached with 30% of added milk with control rumen fluid donors (pH = 5.46) compared with 40% of added milk in Actisaf Sc47 rumen fluid donors (pH = 5.49). To estimate the effect of RD on dry matter (DM) and neutral detergent fiber (NDF) degradability, we used 4 Daisy jars. Each jar received either control or Actisaf Sc47 rumen fluid with or without addition of milk. Within each jar, 3 Ankom F57 bags containing 500 mg of wheat straw were incubated for 48 h. Without addition of milk, DM degradability was 28.14% and 33.96% for the control and experimental group, respectively ($P = 0.06$), whereas values for NDF degradability were 21.55% and 29.44% ($P = 0.044$). With the addition of milk, DM degradability was 22.53% and 24.58% for the control and experimental group, respectively ($P = 0.2$), whereas the corresponding values for NDF degradability were 17.03% and 19.35% ($P = 0.4$). The use of Actisaf Sc 47 attenuated the negative effects of RD in calves. The Ankom and Daisy incubator systems appear to be practical tools for simulating RD and screening different additives.

Key Words: in vitro, probiotic, rumen drinking

2806 Synergy of essential oils and fumaric acid differentially modulates rumen microbiome in dairy cows. J. O. Alabi*, K. A. Ike, A. E. Kholif, and U. Y. Anele, *North Carolina A&T State University, Greensboro, NC*.

Previous findings from our group showed that essential oil blend (EOB) combined with fumaric acid (FA) can modulate ruminal fermentation and mitigate greenhouse gas emissions in dairy cows. This study aimed at investigating the rumen microbiome of nonlactating Holstein Friesian cows using a rumen simulation technique system. Three distinct EOB were evaluated: GCLN (garlic, lemongrass, cumin, lavender, and nutmeg; 4:2:2:1:1); ACOG (anise, clove, oregano, cedarwood, and ginger; 4:2:2:1:1); and CAPO (clove, anise, peppermint, and oregano; 4:3:2:1). The total mixed ration (TMR) contained corn silage (60%), alfalfa hay (20%), and a concentrate mix (20%). Sixteen fermentation chambers were allotted to 4 treatments, each with 4 replicates, following a completely randomized design that lasted for 9 d. Treatments evaluated were Control (TMR), GCLN, ACOG, and CAPO. Dietary treatments contained EOB (10 μ L/g) and FA (30 mg/g) added to the TMR. Rumen fluids were sampled for 5 d following 4 d of adaptation. Rumen

fermentation data were analyzed with PROC GLM in SAS. Amplicon sequencing data from Illumina NextSeq 2000 were processed using QIIME2 (v2022.2) for analysis and the SILVA database for taxonomic classification. Only GCLN reduced ($P < 0.05$) CH₄ emissions by 60.2%. Compared with the control, GCLN differentially ($P < 0.05$) decreased the abundance of *Bacillus*, *Serratia*, *Escherichia-Shigella*, *Bacteroides*, *Catenisphaera*, and *Pseudobutyrvibrio* and increases the abundance of *Clostridium sensu stricto 1*, *Anaeroplasma*, *Victivallaceae*, *Endomicrobium*, *Fibrobacter* and *Eubacterium ventriosum* group. ACOG reduced ($P < 0.05$) the abundance of *Sharpea*, *Rummeliibacillus*, *Chryseobacterium*, *Catenibacterium* and *Pseudobutyrvibrio* and increased that of *Vagococcus*, *Succinimonas*, *Clostridium sensu stricto 1*, *Campylobacter*, *Endomicrobium*, *Eubacterium ventriosum* group and *Succinivibrio*. CAPO differentially ($P < 0.05$) increased *Succinimonas*, *Gastranaerophilales*, *Campylobacter*, *Enterococcus*, *Firmicutes*, *Endomicrobium*, *Elusimicrobium*, *Puniceicoccaceae*, and *Succinivibrionaceae* and decreased *Erysipelotrichaceae* UCG 002, *Rummeliibacillus*, *Sharpea*, *Coriobacteriaceae*, *Paracoccus*, *Prevotellaceae* NK3B31 group, *Prevotellaceae* UCG 004, *Chryseobacterium*, *Bacillus*, and *Kurthia*. The random forest classification showed the lower relative abundance of *Cyanobacteria*, *Elusimicrobiota*, and *Verrucomicrobiota*. In conclusion, essential oil blends and fumaric acid significantly influenced the rumen microbiome in a way that modified fermentation dynamics, but only GCLN was able to reduce methane gas production through changes in microbial community dynamics.

Key Words: microbiome, essential oil, dairy cow

2807 Microbes mostly contributing to methionine in rumen microbial protein and their association with feed efficiency. H. F. Monteiro^{*1}, R. Profeta¹, B. C. Weimer¹, C. C. Figueiredo², J. E. P. Santos³, R. S. Bisinotto⁴, E. S. Ribeiro⁵, F. Peñagaricano⁶, A. P. Faciola³, and F. S. Lima¹, ¹Department of Population Health and Reproduction, University of California-Davis, Davis, CA, ²Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA, ³Department of Animal Sciences, University of Florida, Gainesville, FL, ⁴Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL, ⁵Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, ⁶Department of Animal and Dairy Sciences, University of Wisconsin-Madison, Madison, WI.

Our group has previously demonstrated how an ultra-deep metatranscriptomic analysis of rumen content may be used as a proxy to comprehensively characterize the AA profile in rumen microbial protein (MP) from dairy cows. Considering that each microbial species has a distinct AA content, we hypothesized that the microbes thriving in feed-efficient cows are the predominant microbes making the top 1% of microbes with the greatest methionine (Met) concentration in their proteins among all MP in the rumen. The goal of the study was to use de novo protein characterization and taxonomy annotation of proteins to rank microbes with the greatest Met concentration and evaluate their association with feed efficiency, given that Met is a limiting AA to dairy cows. From a cohort of 454 lactating cows phenotyped for feed efficiency, 24 cows were matched by site, diet (CP: 17% $\pm$ 0.88%), parity, body weight, energy balance, and milk production by DMI (26.1 vs. 20.7 kg/d) were used for ultra-deep dual-RNA sequencing analysis of the rumen content (oroesophageal tubing collection 2–6 h after feeding). De novo gene assembly was performed in *Trinity*, and their

abundance was calculated using *Salmon*. A peptide translation prediction was performed in *Transdecoder*. To assign microbial taxonomy to proteins, all genes were aligned against the nt database from NCBI using BLASTn. Then, the proteins accounting for the top 1% of proteins with the greatest Met content were selected for comparisons between the least and most feed-efficient cows. From 46,030 proteins with the greatest Met concentration (average Met = 7.93% $\pm$ 1.49% SD), 16,465 were successfully assigned to a microbial taxa (average Met = 8.02% $\pm$ 1.50% SD). *Prevotella* (20%), *Xylanibacter* (7%), and *Segatella* (4%) made up the top 3 microbes from all the top 1% proteins with greatest Met, with emphasis on *Xylanibacter ruminicola*, which had 21% of all proteins annotated up to the species level in this group. Interestingly, these microbes, especially *Xylanibacter ruminicola*, were the main microbes associated with improved feed efficiency in the study and could be potentially proposed as (auto)probiotics. This study highlights how microbial changes in dairy cows may also be affecting the AA profile of rumen MP and, consequently, production traits.

Key Words: metatranscriptomics, microbiome, probiotic

2808 Determining N losses through enteric ammonia emission in lactating cows. B. Bae^{*} and C. Lee, Department of Animal Sciences, The Ohio State University, Wooster, OH.

In N balance studies, overestimations of N retention have been reported (5%–10% of N intake) in multiparous lactating cows. The sources of overestimation are not clear. Therefore, we assessed potential N losses through enteric NH₃ emission with 2 experiments. Experiment 1 was conducted to assess a diurnal pattern of enteric NH₃ emission with 8 midlactation Holstein cows in a crossover design (different fatty acids for dietary treatments). Experiment 2 was conducted to assess the effects of dietary CP on enteric NH₃ emissions with 16 midlactation Holstein cows in a randomized complete block design. Cows were fed diets with 2 levels of CP (15.5% or 17.1% CP of DM). Each period of Exp. 1 lasted for 21 d, and enteric gas was measured at 8 time points over the last 3 d (every 3 h sampling in a 24-h cycle). Exp. 2 lasted for 21 d, and enteric gas was measured at 5 time points over 3 d (1, 6, 9, 15, and 18 h after feeding). We developed a face-mask system and used it for enteric gas measurements. Data from Exp. 1 were analyzed using PROC MIXED in SAS, with period and cow within block as random effects and diet, time, and their interaction as fixed effects. Data from Exp. 2 were analyzed with block as a random effect and CP level as a fixed effect. In Exp. 1, enteric NH₃ emission (0.82 g/d) was not affected by diet and showed no interaction of diet $\times$ time. However, a diurnal pattern was observed (time $P < 0.01$) where enteric NH₃ decreased immediately after feeding, gradually increased until 6 h after feeding, and then remained until the next feeding time. In Exp. 2, although N intake was greater ($P < 0.01$) for cows on high CP than low CP, enteric NH₃ emission did not differ (0.61 g/d). Data in Exp. 1 and 2 were combined, and N intake was regressed on enteric NH₃-N emission, and results showed no linear relationship ($P = 0.41$; $r^2 = 0.05$; RMSE = 91.8; CCC = 0.11). In conclusion, enteric NH₃ emissions had a diurnal pattern that likely occurred due to pH changes in the rumen. However, enteric NH₃ emission was not affected by dietary CP or N intake. Although N losses through enteric NH₃ emission were detected, the losses were less than 0.1% of N intake and were ignorable.

Key Words: enteric gas, face mask, nitrogen

Author Index

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