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Carbohydrate nutrition associated with health of overwintering honey bees

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In temperate climates, honey bees rely on stored carbohydrates to sustain them throughout the winter. In nature, honey serves as the bees' source of carbohydrates, but when managed, beekeepers often harvest honey and replace it with cheaper, artificial feed. The effects of alternative carbohydrate sources on colony survival, strength, and individual bee metabolic health are poorly understood. We assessed the impacts of carbohydrate diets (honey, sucrose syrup, high-fructose corn syrup, and invert syrup) on colony winter survival, population size, and worker bee nutritional state (i.e., fat content and gene expression of overwintered bees and emerging callow bees). We observed a nonsignificant trend for greater survival and larger adult population size among colonies overwintered on honey compared to the artificial feeds, with colonies fed high-fructose corn syrup performing particularly poorly. These trends were mirrored in individual bee physiology, with bees from colonies fed honey having significantly larger fat bodies than those from colonies fed high-fructose corn syrup. For bees fed honey or sucrose, we also observed gene expression profiles consistent with a higher nutritional state, associated with physiologically younger individuals. That is, there was significantly higher expression of vitellogenin and insulin-like peptide 2 and lower expression of insulin-like peptide 1 and juvenile hormone acid methyltransferase in the brains of bees that consumed honey or sucrose syrup relative to those that consumed invert syrup or high-fructose corn syrup. These findings further our understanding of the physiological implications of carbohydrate nutrition in honey bees and have applied implications for colony management.

Key words: Apis mellifera, nutrition, metabolic health, overwintering, carbohydrate, sugar

Introduction

Honey bees are an important managed pollinator in many cropping systems (Calderone 2012). Since the mid-2000s, there has been recognition of unacceptably high rates of colony loss, with particularly high losses over the winter (>30% annually across the United States) (Cox-Foster et al. 2007, vanEngelsdorp et al. 2008, vanEngelsdorp and Meixner 2010), and beekeepers continue to report comparably low winter survival in recent years (Bruckner et al. 2023). Despite the high rate of colony loss, the total number of managed honey bee colonies has increased globally over the last half century (Phiri et al. 2022), as beekeepers are capable of creating new colonies by splitting surviving colonies (vanEngelsdorp and Meixner 2010). However, colony replenishment represents a significant economic cost to the beekeeper (Bixby et al. 2023). Additionally, while the absolute number of colonies has increased, the need for managed pollinators in agricultural production has simultaneously increased at a higher rate, creating a deficit between supply and demand for pollination services (Aizen and Harder 2009).

Many factors and their interactive effects have been associated with increased rates of colony loss including pesticides, pathogens, parasites, and poor nutrition (Potts et al. 2010, vanEngelsdorp and Meixner 2010, Goulson et al. 2015). While several of these are environmental stressors, largely beyond the control of the beekeeper, beekeepers can manage colony nutrition in part by supplementing feed (Standifer et al. 1977). Particularly in winter, food availability (namely, carbohydrate sources) can be regulated by the beekeeper. Beekeepers can either leave stored honey for the bees to consume or extract the honey and replace it with another carbohydrate source (reviewed in Brodschneider and Crailsheim 2010). Providing ample sources of carbohydrates over the winter is essential to winter survival, as carbohydrates provide energy for thermoregulation (Heldmaier 1987) and proper immune function (Cotter et al. 2011, DeGrandi-Hoffman and Chen 2015). Beyond a simple source of carbohydrates, honey also contains several secondary compounds that can have antimicrobial properties and function to reduce oxidative stress (Berenbaum and Calla 2021).

Beekeepers have many options for sources of carbohydrate feed, each with potential tradeoffs. Sucrose syrup, high-fructose corn syrup (HFCS), and invert syrup are common artificial feeds because these carbohydrate sources are inexpensive, easy to obtain, and readily eaten by the bees. Invert syrup is commercially prepared from sucrose syrup through the action of the enzyme invertase, which breaks sucrose down into glucose and fructose, creating a product similar to honey in a mono-carbohydrate profile (Table 1) (Potter and Hotchkiss 1998). Invert syrup is as viscous as honey and is less likely to ferment or crystallize (Potter and Hotchkiss 1998), making it an appealing option over sucrose syrup or HFCS. Sucrose syrup is prone to fermentation and mold, and HFCS can become toxic to bees due to the formation of hydroxymethylfurfural (HMF) if it is not stored properly (LeBlanc et al. 2009). Hydroxymethylfurfural can also form in improperly prepared invert syrup, and the addition of acids to catalyze the invert syrup conversion can directly increase bee mortality (Frizzera et al. 2020).

Previous studies have examined the effects of artificial carbohydrate supplementation on colony and individual bee health, often observing no difference among feed types. A study by Barker and Lehner (1978) found that caged worker bees fed sucrose syrup had longer survival than those fed honey or HFCS (Barker and Lehner 1978). But at the colony level, a comparison of sucrose syrup to HFCS showed no difference in weight gain, honey production, cluster size, and worker weight (Severson and Erickson 1984). Likewise, Brodschneider et al. (2010) compared sucrose syrup, invert syrup, and starch syrup and found no difference in colony overwintering mortality in Austria. In the temperate climate of North Carolina, Harris et al. (2011) studied various winter feeds (6 different sucrose and HFCS dilutions/blends) and reported excellent survival among all feeds tested, even among *Nosema*-infected colonies. One study by Sammataro and Weiss (2013) found greater performance in colonies fed sucrose syrup compared to HFCS, but this study did not compare results to honey, the original overwintering food for honey bees. Given that beekeeper reports and empirical surveys still rank starvation as a primary driver of winter loss (Brodschneider et al. 2010, Bruckner et al. 2023), there is a need to continue investigating how natural (honey) and artificial supplemental diets affect colony and individual bee winter survival and condition in a field-realistic setting (Gregorc et al. 2019). In this study, we set up a randomized feeding experiment in the field, coupled with physiological analysis (i.e., measurement of worker lipid content and brain expression levels of health-associated genes) to determine the effects of artificial feeds and honey on overwintering survival and worker honey bee health.

Materials and Methods

Colony Management and Evaluation

In August 2020, 4 beekeepers from Pennsylvania, USA, were solicited as participants. Each participant chose 12 healthy colonies, kept in the same apiary, of approximately the same size (based on a cluster count [Nasr et al. 1990]) to participate in the study. In each bee yard, 3 colonies (1/4) served as the control where honey was not harvested but left in the hive for the colony to consume during the winter. For the remaining colonies, capped honey was harvested in July 2020, according to the usual beekeeping practices. All colonies were treated for parasitic Varroa mites (*Varroa destructor*) in the second half of July using Formic Pro (NOD Apiary Products, Ltd.) to keep mite levels below a 2% infestation threshold (Jack and Ellis 2021), but data on specific mite infestation rates were not collected. In early September, colonies that had honey removed were given a medium super with drawn comb (built-out wax cells) and fed one of 3 diets (3 colonies per diet, per apiary): (i) 2:1 sucrose syrup, (ii) high-fructose corn syrup 55 (HFCS), or (iii) invert syrup (Pro Sweet; Mann Lake Ltd.). Up to 5 gallons of feed was added to each colony to ensure a minimum of 27 kg of stored food prior to November.

In March 2021, after wintering, colony survival was assessed, and surviving colonies were given an ordinal strength measurement (≤3 frames = small, 4–6 frames = medium, ≥7 frames = large) based on the adult population cluster size (Nasr et al. 1990).

Worker Bee Collection

Individual bee sampling was done in March 2021 after brood rearing had resumed and before spring brood eclosed (Seeley and Visscher 1985). By choosing this narrow time window, we were able to collect both overwintered worker bees (wintered workers) that survived since fall 2020 on the feed provided, as well as newly emerging bees (callows) that had not directly consumed the provided feed.

Wintered workers from surviving colonies were scooped off the frames into 50-ml plastic tubes (Globe Scientific Inc.), and callows near eclosion were pulled out of cells using forceps after uncapping and visually confirming maturity (eyes fully developed, antennae, and mouthparts moving) and placed into plastic bags. All samples were immediately placed on dry ice in an insulated container in the field, followed by storage in a −80 °C freezer the same day. Samples designated for gene expression analysis were placed in a microtube with 1 ml of RNAlater-ICE reagent (Ambion Life Technologies) and kept at −20 °C for 2 days to allow penetration of the reagent into the tissue. Brains were then dissected and stored at −80 °C until RNA extraction.

Lipid Extraction

In insects, the fat body is the site of nutrient storage (Arrese and Soulages 2010), and in honey bees, the size of the fat body is considered a bioindicator of nutritional state (Alaux et al. 2017), physiological age, task (Toth and Robinson 2005), and summer versus winter state (Döke et al. 2019). The fat body is also the site of vitellogenin synthesis, which plays a key role in the regulation of aging, immunocompetence, and survival (Seehuus et al. 2006, Amdam 2011). To assess this biomarker, we performed lipid extraction on a total of 120 wintered bees. These 120 bees were from 12 colonies, 3 colonies per feed type, and 9, 10, or 11 bees per colony (for a total of 30 bees per feed type). Due to poor survival, colonies

Table 1. Percent of sugar content of each type of sugar in honey and 3 artificial feeds used to provide honey bees with winter feed. Carbohydrate composition of honey and high fructose corn syrup (HFCS 55) is based on information from the literature

	Honey (White and Doner 1980)	Invert syrup (Mann Lake Ltd., 2023)	HFCS 55 (Hanover and White 1993)	Sucrose syrup (as prepared)
Fructose	38%	22%	55%	0%
Glucose	31%	27%	42%	0%
Sucrose	1.3%	50%	0%	100%
Maltose	7.3%	0.5%	0%	0%
Other	22.4%	0.5%	3%	0%

Table 2. Primer list. Primers for genes associated with development in honey bees and housekeeping gene provided along with their GenBank accession numbers, melting temperatures (Tm) used, and calculated primer efficiencies

Target gene	Gene description	GenBank access number	Primer sequence forward and reverse	Tm (°C)	% Efficiency
Jhamt	Juvenile hormone acid methyltransferase	JQ858262.1	TTGGACATAGGTTGCGGACC AATCCTTTTCCTCCTGGCCG	57	95.84
Vg	Vitellogenin	NP_001011578	AGTTCCGACCGACGACG TTCCCTCCCACGGAGTCC	57	96.73
Ilp1	Insulin-like peptide 1	GB17332	CGATAGTCCTGGTCGGTTTG CAAGCTGAGCATTGCAC	55	97.93
Ilp2	Insulin-like peptide 2	GB10174	TTCCAGAAATGGAGATGGATG TAGGAGCGCAACTCCTCTGT	52	98.46
RPL32	Ribosomal protein L32	NM_001011587	TGTGCTGAAATTGCTCATGG CGTAACCTTGCACTGGCATA	55	101.88

were not evenly stratified across apiaries (8 colonies were sampled in one apiary, 2 colonies were sampled in a second apiary, and 1 colony each was sampled from the remaining 2 apiaries).

Fat extraction was performed on eviscerated abdomens, as described in O'Donnell and Jeanne (1995) and modified for honey bees (Ortiz-Alvarado et al. 2020). The eviscerated abdomens were placed in a drying oven at 70 °C for 3 days. Dry weight was obtained using a Fisher 11 analytical balance (Fisher Scientific, Hampton, NH, USA) accurate to 0.1 mg. Dried abdomens were then placed in 5 ml of extraction solution (2:1 chloroform: methanol; Sigma–Aldrich) on a rotary shaker (Thomas Scientific, Swedesboro, NJ, USA) for 3 days. The extraction solution was replaced every 24 h for 3 days. After the extraction period, abdomens were placed in the drying oven at 70 °C for 2 days. Extracted weight was obtained after the second drying period, and total lipid content was calculated by subtracting the initial dry weight and the extracted dry weight of each abdomen.

Gene Expression

To determine the effect of feed type on gene expression, we chose targets related to development and nutrition. Juvenile hormone (*JH*) and vitellogenin (*Vg*) function as indicators of development rate, longevity, nutritional status, and stress and disease resistance (reviewed Amdam 2011). Likewise, insulin-like peptides 1 and 2 (*Ilp1* and *Ilp2*, respectively) are involved in nutrition, metabolism, stress, and aging (Ament et al. 2008, 2011, Nilsen et al. 2011, Ortiz-Alvarado et al. 2020), with *Ilp*1 being more highly expressed in the brains of older bees and/or colonies experiencing poor nutrition (Corona et al. 2007, Ament et al. 2008). While the factors affecting the expression of *Ilp*2 are less consistent and clear, *Ilp2* may be more highly expressed in young, well-fed bees and is correlated with *Vg* expression (Amdam 2011, Nilsen et al. 2011). Levels of JH were determined indirectly by measuring juvenile hormone acid methyltransferase (*Jhamt*). Jhamt is an enzyme that catalyzes the production of JH from JH precursors (Shinoda and Itoyama 2003, Minakuchi et al. 2008).

Primers for *Jhamt*, *Vg*, *Ilp1*, *Ilp2*, and reference gene *RPL32* were obtained from the literature from experiments related to development and nutrition in honey bees (Corona et al. 2007, Scharlaken et al. 2008, Ortiz-Alvarado and Giray 2022). The list of target genes, accession numbers, primer sequence, melting temperature (Tm), and percent efficiency is shown in Table 2.

RNA was extracted from a total of 39 ($N_{callows}$ = 19, $N_{wintered}$ = 20) brain tissues. Ten bees each were sampled from colonies fed honey, HFCS, and invert syrup, and 9 bees were sampled from the sucrose syrup treatment. Of these ~10 bees per feed treatment, half were wintered and half were callow. Sampled bees were from 22 different colonies, unequally stratified across the 4 apiaries, and 1–4 bees were sampled per colony (Supplementary Table 1).

Brains were dissected in dry ice and homogenized using a TissueLyzer (Qiagen, Hilden, Germany) with 3mm stainless steel beads in a 2-ml round-bottom microtube. RNA was extracted using the EZ1 RNA Tissue Mini Kit (Qiagen) and automatically processed in an EZ1 Advanced XL instrument (Qiagen). RNA samples were quantified in a QubitTM (Invitrogen, Waltham, MA, USA) with a high-sensitivity RNA assay kit. Following RNA isolation and quantification, samples were normalized to a concentration of 2 µg/µl. Total RNA (200 ng) from samples was reverse-transcribed using the iScript Reverse Transcription Supermix for RT-qPCR (Bio-Rad, Hercules, CA, USA) following the manufacturer's protocol.

Quantitative PCR (qPCR) analysis was performed using the primers listed in Table 2, in a QuanstudioTM 3 Real-Time PCR System (Thermo Fisher, Waltham, MA, USA) following the standard protocol for 40 cycles; denature at 95 °C for 10 s, annealing at primer Tm for 30 s and elongation at 72 °C for 15 s × 40, with postamplification melt curve analysis. Ribosomal protein L32 (*RPL32*) was used as a reference gene (Scharlaken et al. 2008) for standard quantification purposes. Primer efficiency was measured using the standard curve analysis method using pooled samples in five 1:10 dilutions (Larionov et al. 2005). Primer efficiency was then calculated using the qpcR package (v. 1.4-1) (Li et al. 2022) R (R Core Team 2022). qPCR reactions were prepared with 1 µl of cDNA as a template in a master mix of 1 µl of primers at [10 nM] (1 µl forward and 1 µl reverse primers), 5 µl of iTaq Universal SYBR Green Supermix (Bio-Rad) and 2 µl of water for a final volume of 10 µl. Gene expression was calculated using the ΔΔCt method (Schmittgen and Livak 2008).

Statistical Analysis

All statistical analysis was conducted in R version 4.2.1. Treatment differences in colony survival were analyzed using a generalized linear mixed effects model (GLMM) with the lme4 package (Bates et al. 2015). For the survival model, feed treatment was treated as a fixed effect and apiary was treated as a random effect, specifying a binomial family. Overall analysis of variance was assessed using the car package (Fox and Weisberg 2019), and pairwise comparisons were made using the emmeans package (Lenth 2022). Of the colonies that survived, differences in size (ordinal, 1–3) were analyzed using a cumulative link mixed model with the ordinal package (Christensen 2019), specifying feed type as a fixed effect and apiary as a random effect. To analyze differences in abdominal lipid content of wintered workers (square root transformed), we used a GLMM with feed treatment as the fixed effect and colony as a random effect.

We used the lme4 and emmeans packages to run the GLMM and pairwise comparison, respectively. Differences in gene expression among feed treatments within bee type (wintered vs. callow) were likewise assessed using GLMM with the lme4 package. Relative gene expression data ($2^{-\Delta\Delta Ct}$ values) were log-transformed (excepting our housekeeping gene) and regressed against feed type, with colony as a random effect. To assess pairwise comparisons among feed treatments in gene expression, we used the emmeans package.

Results

Of the 48 colonies assessed in this study, 24 (50%) survived the winter of 2020–2021 (Supplementary Table 2). There was no difference in survival among feed treatments (χ^2_3 = 3.87, df = 3, P = 0.28), and no significant pairwise comparisons between treatments. While not statistically significant, colonies which kept their honey had the highest survival rates (66.7%), followed by those fed invert syrup (58.3%), sucrose syrup (41.7%), and high-fructose corn syrup (33.3%). Of the colonies which survived, feed type was not associated with ordinal colony size at the March assessment ($\lambda_{LR(3)}$ = 3.09, P = 0.38). Like survival, however, colonies fed honey over the winter tended to be larger on average (ordinal size of 2.6 on a 1–3 scale), followed by those fed sucrose syrup (mean score = 2.3), invert syrup (mean score = 2.2), and high-fructose corn syrup (mean score= 1.8).

There were significant differences among feed treatments in wintered worker bee lipid content ($F_{3,7.98}$ = 7.11, P = 0.01). Lipid content ranged from 0.02 to 15.57 mg per bee, averaging 3.54 mg per bee. Pairwise comparisons among diets showed larger fat bodies among bees from colonies fed honey or invert syrup and smaller fat bodies among bees from colonies fed HFCS (Fig. 1).

There were significant differences in gene expression among diets for *Jhamt* (callow: $F_{3, 8.36}$ = 19.85, P < 0.01; wintered: $F_{3, 10.36}$ = 63.42, P < 0.01), *Vg* (callow: $F_{3, 10.00}$ = 21.68, P < 0.01; wintered: $F_{3, 10.60}$ = 37.75, P < 0.01), *Ilp1* (callow: $F_{3, 7.22}$ = 23.00, P < 0.01; wintered: $F_{3, 9.29}$ = 46.32, P < 0.01), and *Ilp2* (callow: $F_{3, 10.00}$ = 14.06, P < 0.01; wintered: $F_{3, 9.58}$ = 4.73, P = 0.03). Generally, higher expression of *Vg* and *Ilp2* was seen among bees from colonies fed sucrose syrup, while colonies fed HFCS or invert syrup had higher expression of *Jhamt* and *Ilp1* (Fig. 2). There was also some evidence of higher expression of *Vg* and *Ilp2* among bees from honey-fed colonies, compared with those fed HFCS or invert syrup, though in many cases this trend was not significant. Pairwise differences between feed treatments within bee type are shown in Figure 2. Our reference gene, *RPL32* was invariant in its expression among the treatment groups for both the callow ($F_{3, 8.36}$ = 0.81, P = 0.52) and wintered bees ($F_{3, 9.91}$ = 0.99, P = 0.44), making it an appropriate reference gene for normalization (Bustin et al. 2009).

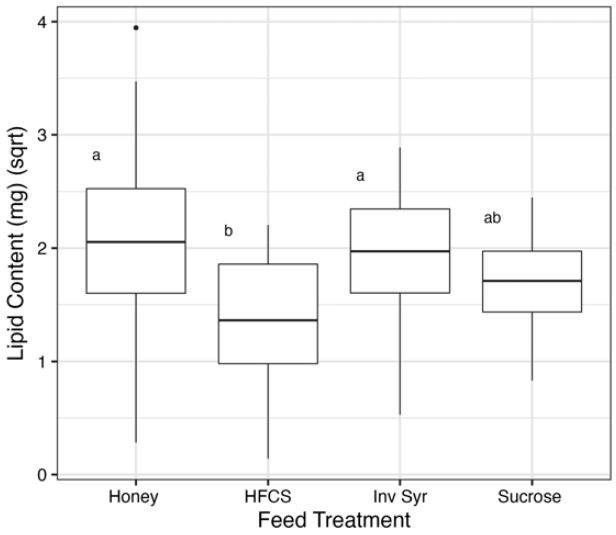

Fig. 1. Boxplot showing the differences in lipid content (fat body size) of overwintered worker bees among honey bee colony feeding treatments. These treatments included feeding honey (Honey), high fructose corn syrup (HFCS), invert syrup (Inv Syr), and sucrose syrup (Sucrose). Significant pairwise comparisons ($\alpha \leq 0.05$) are indicated by different letters.

Discussion

Carbohydrates are the primary source of energy for honey bee workers. In winter, when the colony cannot forage, bees rely primarily on stored food, either honey produced from foraged nectar or other forms of carbohydrate provided by the beekeeper (Seeley and Visscher 1985, Kunert and Crailsheim 1988). In our study, we did not detect a significant effect of feeding honey bee colonies different sources of carbohydrates in fall on overwintering survival or colony size but observed, a nonsignificant trend for higher survival and larger colony size among bees from colonies that fed on honey over the winter. By comparison, colonies fed HFCS had the lowest colony survival and colony size though these effects were not statistically significant. A previous study by Sammataro and Weiss (2013) found that colonies overwintered on HFCS syrup produced less brood in spring, had smaller adult population sizes, produced less wax, and had lighter workers than those overwintered on sucrose syrup. Similarly, Barker and Lehner (1978) found that caged workers survived longest on sucrose solution (LT_{50} = 56.3 days) compared with HFSC (37.7 days) or honey (31.3 days). While these findings seem to suggest sucrose syrup is preferable to HFCS as an artificial feed, other studies have found no adverse effect of feeding HFCS, compared with sucrose syrup or other carbohydrate feeds (Severson and Erickson 1984, Brodschneider et al. 2010). Further work is needed to assess these effects at the colony level and within different contexts (e.g., under stress from pesticides or pests, and in different climates).

At the individual bee level, we observed variation in fat body size and gene expression among bees from colonies fed different carbohydrate diets. Like the trends at the colony level, the largest fat bodies were seen among bees from colonies fed honey and invert syrup, and the smallest were seen among bees from colonies fed HFCS. Bees fed honey and sucrose syrup generally showed higher expression of *Vg* and *Ilp2*, and lower expression of *Jhamt* and *Ilp1*, relative to bees fed HFCS and invert syrup. These gene expression patterns, particularly in sucrose-fed bees are consistent with what would be expected in young, well-fed bees and have been linked to longevity and overwintering survival (Amdam and Omholt 2002, Ament et al. 2008, Amdam 2011, Nilsen et al. 2011, Amdam et al. 2012, Döke et al. 2015, Alaux et al. 2017). While we failed to observe statistically significant effects at the colony level on survival or colony size, the patterns we observed in fat body size and gene expression among individuals could be indicative of colony condition in the near future (Alaux et al. 2011, 2017). Because higher lipid content in worker bees is correlated with longevity and resilience (Amdam and Omholt 2002), these results may offer a mechanistic understanding of how different carbohydrate sources might yield varying success for overwintering survival.

Bees fed invert syrup or HFCS were more similar in their gene expression patterns, among our target group of genes, than those fed sucrose syrup or honey. Expression profiles of honey were largely

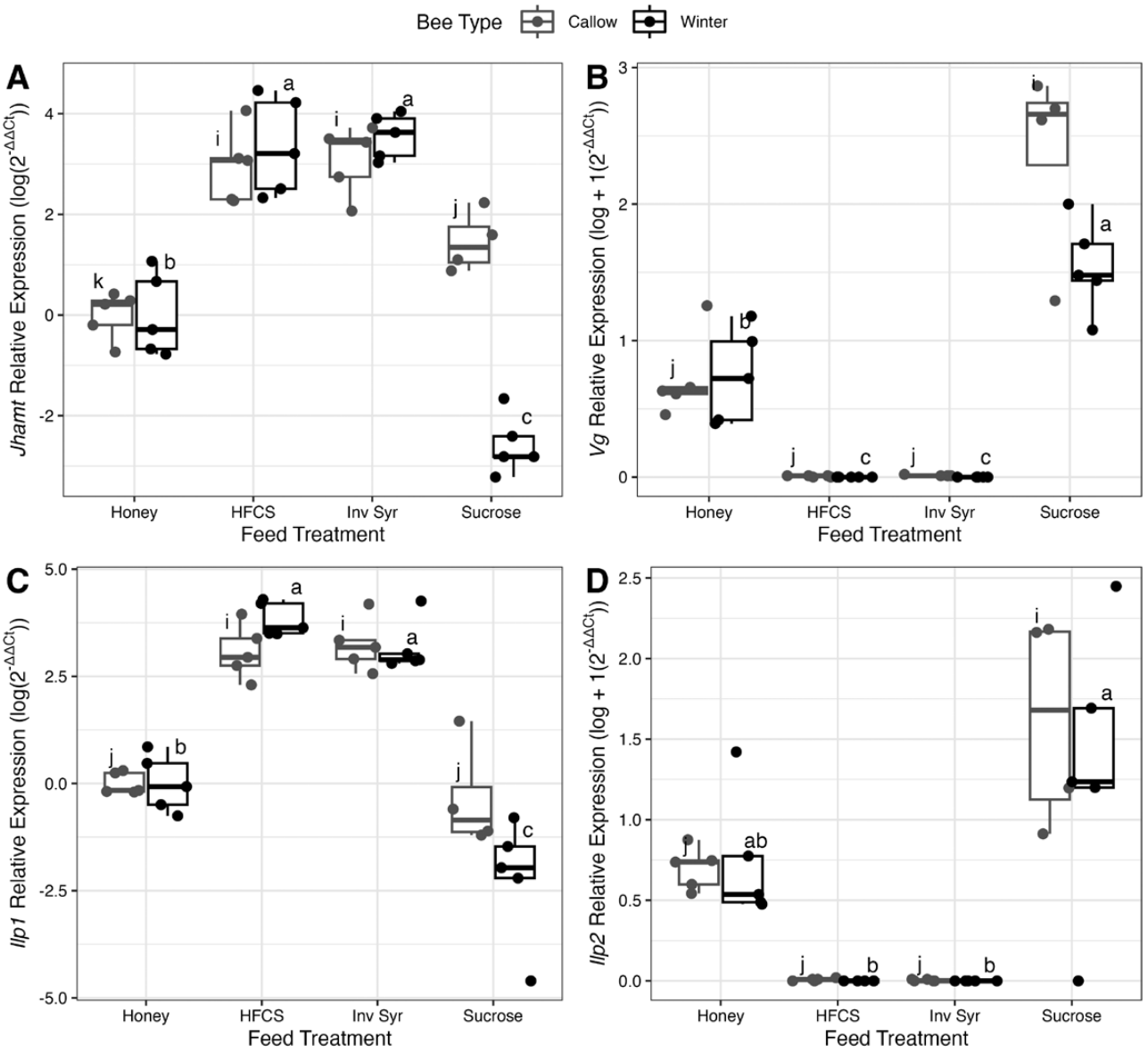

Fig. 2. Relative gene expression of juvenile hormone acid methyltransferase (*Jhamt*) (A), vitellogenin (*Vg*) (B), insulin-like peptide 1 (*Ilp1*) (C), and insulin-like peptide 2 (*Ilp2*) (D) among callow and winter bees from colonies fed different diet treatments, plotted as jittered data points over summary boxplots. Diet treatments included honey (Honey), high fructose corn syrup (HFCS), invert syrup (Inv Syr), and sucrose syrup (Sucrose). Significant pairwise comparisons ($\alpha \leq 0.05$) within bee type (i-j-k for callows, and a-b-c for winter bees) are indicated by different letters.

similar to sucrose but showed some similarities to HFCS and invert syrup (particularly among the *Vg* and *Ilp2* data). This may be explained by the similar carbohydrate composition of honey, invert syrup, and HFCS, compared sucrose syrup (Table 1). However, this finding is somewhat unexpected given that Wheeler and Robinson (2014) analyzed HFCS, sucrose, and honey and found that genome-wide transcriptional patterns were more similar between HFCS and sucrose than either diet to honey. The findings by Wheeler and Robinson suggest that there are transcriptional differences that we did not detect by choosing to target a set of functionally important genes for analysis. These authors also assessed fat body tissue, rather than brains, which could also explain some of the different observations between our two studies. It should be noted that because vitellogenin is not synthesized in the brain, any expression we observed is likely from peripheral fat tissue surrounding the brain (Corona et al. 2007, Münch et al. 2015). Therefore, slight inconsistencies in the dissection technique among samples may have contributed to greater variation in observed *Vg* expression levels. However, the trends we see in *Vg* expression are consistent with *Ilp2* and oppose *Jhamt*, as we would expect (Amdam and Omholt 2003, Corona et al. 2007, Ament et al. 2008, Amdam 2011, Nilsen et al. 2011), providing an added degree of confidence in our results.

Honey is distinct from artificial diets in many ways that could influence gene expression patterns; honey contains trace pollen, minerals, and other compounds (e.g., antimicrobial peptides) that are missing from artificial carbohydrate sources (Berenbaum and Calla 2021). Mao et al. (2013) determined that compounds in honey, including p-coumaric acid, affect the immune system of bees by upregulating detoxification and antimicrobial peptide genes (Mao et al. 2013). Thus, researchers suggest that honey aids in protecting bees because it contains compounds that are missing from other feeds such as sucrose syrup and HFCS (Johnson et al. 2012, Mao et

al. 2013). The lack of compounds such as p-coumaric acid may leave the bees that feed on artificial carbohydrate sources more vulnerable to pesticides and infections (Mao et al. 2013). While we controlled parasite levels among our research colonies, the effects of artificial feed may be even more pronounced when colonies are affected by other stressors. Future studies aimed at assessing these interactive effects would be valuable.

Across each of the carbohydrate diet treatments, winter bees showed similar pairwise differences in gene expression to callow bees. This is remarkable, given the differences in carbohydrate diet quantity and source for each group. Winter bees fed directly on and consumed large quantities of the carbohydrate diets during their adult life. While callow bees, having just emerged, did not directly consume large quantities of the diets. Rather, their gene expression likely reflects what they were fed as developing larvae (small amounts of the diets along with royal jelly produced by nurse bees) (Brodschneider and Crailsheim 2010). Previous studies on the sugar content of royal jelly showed no impact from various carbohydrate feeds (Sesta et al. 2006). So, it is notable that we observed a clear signature of the carbohydrate diets among callows. The callow stage (<24 h) is an understudied period of development. In spring/summer bees, brain gene expression shifts throughout early adult development (<8 days) (Whitfield et al. 2006). Therefore, future studies should continue to examine these effects across, and/or after, early adult developmental stages. It should be noted that while brood rearing typically commences in our study region in late February (Seeley and Visscher 1985), we cannot guarantee that all workers we collected were winter bees (and not abnormally early emerging spring bees). While we feel that this is unlikely, inadvertently collecting early spring bees among our winter worker samples could have led to increased variation in gene expression within this group, and/or contributed to similarities in gene expression between spring callows and winter workers.

Differences in the effects of feeds in this study may relate to the disruptive influence of different sugars on honey bee gut microbiota (Taylor et al. 2019). The honey bee gut microbiome contains a core set of bacteria (Moran 2015), which are capable of digesting and metabolizing plant-based carbohydrates (Lee et al. 2015, Zheng et al. 2019). The microbiome affects the pH of the gut and impacts bee growth, behavior, hormones, and physiology (Zheng et al. 2017). Changes to the core microbiome or antibiotics have a negative impact on bees (Raymann and Moran 2018, Ortiz-Alvarado 2019, Ortiz-Alvarado et al. 2020), making them more susceptible to diseases (Hamdi et al. 2011, Maes et al. 2016) and impacting the expression of developmental genes, including *Vg* (Maes et al. 2016, Ortiz-Alvarado and Giray 2022). While D'Alvise et al. (2018) found that gut microbiota were largely unaffected by winter feed type, recent work by Taylor et al. (2019) found that sucrose-rich diets can alter subcore bacteria in honey bee guts. This highlights carbohydrate influences on the honey bee microbiome as an area for further mechanistic investigation.

Poor colony-level sampling of bees for gene expression analysis is a limitation of our study that may have contributed to greater variation in our expression data and some of the marginal pairwise trends we observed. By stratifying our sampling across colonies (rather than sampling more extensively from fewer colonies), we hoped to expand our scope of inference and obtain more broadly applicable results. While the colony source was statistically accounted for as a random intercept, our results indicate high variability in gene expression among colonies, relative to residual variance. More robust sampling at the colony level could have increased precision and contributed to a higher degree of significance.

Providing a nutritional winter diet may be the key to stress resilience and overwintering success (Brodschneider et al. 2010, Dolezal and Toth 2018). Future work aimed at assessing the season-long impacts of various carbohydrate diets on colony winter survival, productivity, and resilience to other stressors could improve our understanding of carbohydrate nutrition in honey bees. Beekeepers should consider the economic tradeoffs of harvesting honey and replacing it with artificial diets, as our study and others suggest artificial diets may not support colony and individual bee health as well as natural honey.

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Author Contributions

Gabriela Quinlan (Data curation [Equal], Formal analysis [Lead], Methodology [Equal], Visualization [Lead], Writing – original draft [Equal], Writing – review & editing [Lead]), Mehmet Ali Doke (Conceptualization [Equal], Data curation [Equal], Investigation [Equal], Methodology [Equal], Validation [Equal], Writing – original draft [Equal], Writing – review & editing [Equal]), Yarira Ortiz-Alvarado (Data curation [Equal], Investigation [Equal], Methodology [Equal], Validation [Equal], Writing – review & editing [Equal]), Norma Rodriguez-Gomez (Investigation [Equal], Methodology [Equal]), Yılmaz Koru (Investigation [Equal], Methodology [Equal]), and Robyn Underwood (Conceptualization [Equal], Data curation [Equal], Funding acquisition [Lead], Investigation [Equal], Project administration [Lead], Supervision [Lead], Writing – original draft [Equal], Writing – review & editing [Equal])

Supplementary Material

Supplementary material is available at *Journal of Insect Science* online.

References

Aizen MA, Harder LD. The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. Curr Biol. **2009**:19(11):915–918. https://doi.org/10.1016/j.cub.2009.03.071

Alaux C, Allier F, Decourtye A, Odoux J-F, Tamic T, Chabirand M, Delestra E, Decugis F, Le Conte Y, Henry M. A 'Landscape physiology' approach for assessing bee health highlights the benefits of floral landscape enrichment and semi-natural habitats. Sci Rep. **2017**:7:40568. https://doi.org/10.1038/srep40568

Alaux C, Dantec C, Parrinello H, Le Conte Y. Nutrigenomics in honey bees: digital gene expression analysis of pollen's nutritive effects on healthy and *Varroa*-parasitized bees. BMC Genomics. **2011**:12:496. https://doi.org/10.1186/1471-2164-12-496

Amdam GV. Social context, stress, and plasticity of aging. Aging Cell. **2011**:10(1):18–27. https://doi.org/10.1111/j.1474-9726.2010.00647.x

Amdam GV, Fennern E, Havukainen H. Vitellogenin in honey bee behavior and lifespan. In: Galizia CG, Eisenhardt D, Giurfa M, editors. Honeybee neurobiology and behavior: a tribute to Randolf Menzel. Dordrecht (The Netherlands): Springer Netherlands; **2012**. p. 17–29.

Amdam GV, Omholt SW. The regulatory anatomy of honeybee lifespan. J Theor Biol. **2002**:216(2):209–228. https://doi.org/10.1006/jtbi.2002.2545

Amdam GV, Omholt SW. The hive bee to forager transition in honeybee colonies: the double repressor hypothesis. J Theor Biol. **2003**:223(4):451–464. https://doi.org/10.1016/s0022-5193(03)00121-8

Ament SA, Chan QW, Wheeler MM, Nixon SE, Johnson SP, Rodriguez-Zas SL, Foster LJ, Robinson GE. Mechanisms of stable lipid loss in a social insect. J Exp Biol. **2011**:214(Pt 22):3808–3821. https://doi.org/10.1242/jeb.060244

Ament SA, Corona M, Pollock HS, Robinson GE. Insulin signaling is involved in the regulation of worker division of labor in honey bee colonies. Proc Natl Acad Sci USA. **2008**:105(11):4226–4231. https://doi.org/10.1073/pnas.0800630105

Arrese EL, Soulages JL. Insect fat body: Energy, metabolism, and regulation. Annu Rev Entomol. **2010**:55:207–225. https://doi.org/10.1146/annurev-ento-112408-085356

Barker RJ, Lehner Y. Laboratory comparison of high fructose corn syrup, grape syrup, honey, and sucrose syrup as maintenance food for caged honey bees. Apidologie. **1978**:9(2):111–116. https://doi.org/10.1051/apido:19780203

Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. J Stat Soft. **2015**:67:1–48. https://doi.org/10.18637/jss.v067.i01

Berenbaum MR, Calla B. Honey as a functional food for *Apis mellifera*. Annu Rev Entomol. **2021**:66:185–208. https://doi.org/10.1146/annurev-ento-040320-074933

Bixby M, Scarlett R, Hoover SE. Winter mortality, diversification, and self-sufficiency affect honey bee (Hymenoptera: Apidae) colony profit in Canada: a model of commercial Alberta beekeepers. J Econ Entomol. **2023**:116(3):686–696. https://doi.org/10.1093/jee/toad056

Brodschneider R, Crailsheim K. Nutrition and health in honey bees. Apidologie. **2010**:41(3):278–294. https://doi.org/10.1051/apido/2010012

Brodschneider R, Moosbeckhofer R, Crailsheim K. Surveys as a tool to record winter losses of honey bee colonies: a two year case study in Austria and South Tyrol. J Api Res. **2010**:49(1):23–30. https://doi.org/10.3896/ibra.1.49.1.04

Bruckner S, Wilson M, Aurell D, Rennich K, vanEngelsdorp D, Steinhauer N, Williams GR. A national survey of managed honey bee colony losses in the USA: results from the Bee Informed Partnership for 2017–18, 2018–19, and 2019–20. J Api Res. **2023**:62(3):429–443. https://doi.org/10.1080/00218839.2022.2158586

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, *et al*. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. **2009**:55(4):611–622. https://doi.org/10.1373/clinchem.2008.112797

Calderone NW. Insect pollinated crops, insect pollinators and US agriculture: trend analysis of aggregate data for the period 1992–2009. PLoS One. **2012**:7(5):e37235. https://doi.org/10.1371/journal.pone.0037235

Christensen RHB. ordinal: Regression models for ordinal data. R package version 2019.12-10. **2019**. https://CRAN.R-project.org/package=ordinal

Corona M, Velarde RA, Remolina S, Moran-Lauter A, Wang Y, Hughes KA, Robinson GE. Vitellogenin, juvenile hormone, insulin signaling, and queen honey bee longevity. Proc Natl Acad Sci USA. **2007**:104(17):7128–7133. https://doi.org/10.1073/pnas.0701909104

Cotter SC, Simpson SJ, Raubenheimer D, Wilson K. Macronutrient balance mediates trade-offs between immune function and life history traits. Funct Ecol. **2011**:25:186–198. https://doi.org/10.1111/j.1365-2435.2010.01766.x

Cox-Foster DL, Conlan S, Holmes EC, Palacios G, Evans JD, Moran NA, Quan P-L, Briese T, Hornig M, Geiser DM, *et al*. A metagenomic survey of microbes in honey bee colony collapse disorder. Science. **2007**:318(5848):283–287. https://doi.org/10.1126/science.1146498

D'Alvise P, Böhme F, Codrea MC, Seitz A, Nahnsen S, Binzer M, Rosenkranz P, Hasselmann M. The impact of winter feed type on intestinal microbiota and parasites in honey bees. Apidologie. **2018**:49(2):252–264. https://doi.org/10.1007/s13592-017-0551-1

DeGrandi-Hoffman G, Chen Y. Nutrition, immunity and viral infections in honey bees. Curr Opin Insect Sci. **2015**:10:170–176. https://doi.org/10.1016/j.cois.2015.05.007

Döke MA, Frazier M, Grozinger CM. Overwintering honey bees: biology and management. Curr Opin Insect Sci. **2015**:10:185–193. https://doi.org/10.1016/j.cois.2015.05.014

Döke MA, McGrady CM, Otieno M, Grozinger CM, Frazier M. Colony size, rather than geographic origin of stocks, predicts overwintering success in honey bees (Hymenoptera: Apidae) in the Northeastern United States. J Econ Entomol. **2019**:112(2):525–533. https://doi.org/10.1093/jee/toy377

Dolezal AG, Toth AL. Feedbacks between nutrition and disease in honey bee health. Curr Opin Insect Sci. **2018**:26:114–119. https://doi.org/10.1016/j.cois.2018.02.006

Fox J, Weisberg S. An R Companion to Applied Regression, Third Edition. Thousand Oaks (CA): Sage; **2019**. https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Frizzera D, Del Fabbro S, Ortis G, Zanni V, Bortolomeazzi R, Nazzi F, Annoscia D. Possible side effects of sugar supplementary nutrition on honey bee health. Apidologie. **2020**:51:594–608. https://doi.org/10.1007/s13592-020-00745-6

Goulson D, Nicholls E, Botías C, Rotheray EL. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science. **2015**:347(6229):1255957. https://doi.org/10.1126/science.1255957

Gregorc A, Sampson B, Knight PR, Adamczyk J. Diet quality affects honey bee (Hymenoptera: Apidae) mortality under laboratory conditions. J Api Res. **2019**:58(4): 492–493. https://doi.org/10.1080/00218839.2019.1614736

Hamdi C, Balloi A, Essanaa J, Crotti E, Gonella E, Raddadi N, Ricci I, Boudabous A, Borin S, Manino A, *et al*. Gut microbiome dysbiosis and honeybee health. J Appl Entomol. **2011**:135:524–533. https://doi.org/10.1111/j.1439-0418.2010.01609.x

Hanover LM, White JS. Manufacturing, composition, and applications of fructose. Am J Clin Nutr. **1993**:58(5 Suppl):724S–732S. https://doi.org/10.1093/ajcn/58.5.724S

Harris B, Harris J, Holden M, Tarpy DR. Impact of supplemental feed type on winter survival of honey bee colonies. (NC): SARE; **2011**. https://projects.sare.org/sare_project/fs11-252/

Heldmaier G. Temperature control in honey bee colonies. BioScience. **1987**:37:395–399. https://doi.org/10.2307/1310562

Jack CJ, Ellis JD. Integrated pest management control of *Varroa destructor* (Acari: Varroidae), the most damaging pest of (*Apis mellifera* L. (Hymenoptera: Apidae)) colonies. J Insect Sci. **2021**:21(5):6. https://doi.org/10.1093/jisesa/ieab058

Johnson RM, Mao W, Pollock HS, Niu G, Schuler MA, Berenbaum MR. Ecologically appropriate xenobiotics induce cytochrome P450s in *Apis mellifera*. PLoS One. **2012**:7(2):e31051. https://doi.org/10.1371/journal.pone.0031051

Kunert K, Crailsheim K. Seasonal changes in carbohydrate, lipid and protein content in emerging worker honeybees and their mortality. J Api Res. **1988**:27(1):13–21. https://doi.org/10.1080/00218839.1988.11100775

Larionov A, Krause A, Miller W. A standard curve based method for relative real time PCR data processing. BMC Bioinf. **2005**:6:62. https://doi.org/10.1186/1471-2105-6-62

LeBlanc BW, Eggleston G, Sammataro D, Cornett C, Dufault R, Deeby T, St Cyr E. Formation of hydroxymethylfurfural in domestic high-fructose corn syrup and its toxicity to the honey bee (*Apis mellifera*). J Agric Food Chem. **2009**:57(16):7369–7376. https://doi.org/10.1021/jf9014526

Lee FJ, Rusch DB, Stewart FJ, Mattila HR, Newton ILG. Saccharide breakdown and fermentation by the honey bee gut microbiome. Environ Microbiol. **2015**:17(3):796–815. https://doi.org/10.1111/1462-2920.12526

Lenth R. _emmeans: Estimated Marginal Means, aka Least-Squares Means_. R package version 1.7.5. **2022**.

Li X, Wang Y, Li J, Mei X, Liu Y, Huang H. qPCRtools: an R package for qPCR data processing and visualization. Front Genet. **2022**:13:1002704. https://doi.org/10.3389/fgene.2022.1002704

Maes PW, Rodrigues PAP, Oliver R, Mott BM, Anderson KE. Diet-related gut bacterial dysbiosis correlates with impaired development, increased mortality and Nosema disease in the honeybee (Apis mellifera). Mol Ecol. **2016**:25(21):5439–5450. https://doi.org/10.1111/mec.13862

Mao W, Schuler MA, Berenbaum MR. Honey constituents up-regulate detoxification and immunity genes in the western honey bee *Apis mellifera*. Proc Natl Acad Sci USA. **2013**:110(22):8842–8846. https://doi.org/10.1073/pnas.1303884110

Minakuchi C, Namiki T, Yoshiyama M, Shinoda T. RNAi-mediated knockdown of juvenile hormone acid O-methyltransferase gene causes precocious metamorphosis in the red flour beetle *Tribolium castaneum*. FEBS J. **2008**:275(11):2919–2931. https://doi.org/10.1111/j.1742-4658.2008.06428.x

Moran NA. Genomics of the honey bee microbiome. Curr Opin Insect Sci. **2015**:10:22–28. https://doi.org/10.1016/j.cois.2015.04.003

Münch D, Ihle KE, Salmela H, Amdam GV. Vitellogenin in the honey bee brain: Atypical localization of a reproductive protein that promotes longevity. Exp Gerontol. **2015**:71:103–108. https://doi.org/10.1016/j.exger.2015.08.001

Nasr ME, Thorp RW, Tyler TL, Briggs DL. Estimating honey bee (Hymenoptera: Apidae) colony strength by a simple method: measuring cluster size. J Econ Entomol. **1990**:83(3):748–754. https://doi.org/10.1093/jee/83.3.748

Nilsen K-A, Ihle KE, Frederick K, Fondrk MK, Smedal B, Hartfelder K, Amdam GV. Insulin-like peptide genes in honey bee fat body respond differently to manipulation of social behavioral physiology. J Exp Biol. **2011**:214(Pt 9):1488–1497. https://doi.org/10.1242/jeb.050393

O'Donnell S, Jeanne RL. Worker lipid stores decrease with outside-nest task performance in wasps: implications for the evolution of age polyethism. Experientia. **1995**:51:749–752. https://doi.org/10.1007/bf01941274

Ortiz-Alvarado Y. Antibiotics effects on metabolism, behavioral development and associated gene expression of honey bee (*Apis mellifera*) [PhD dissertation]. [Rio Piedras (Puerto Rico)]: University of Puerto Rico; **2019**.

Ortiz-Alvarado Y, Clark DR, Vega-Melendez CJ, Flores-Cruz Z, Domingez-Bello MG, Giray T. Antibiotics in hives and their effects on honey bee physiology and behavioral development. Biol Open. **2020**:9(11):bio053884. https://doi.org/10.1242/bio.053884

Ortiz-Alvarado Y, Giray T. Antibiotics alter the expression of genes related to behavioral development in honey bees (Hymenoptera: Apidae). J Insect Sci. **2022**:22(2):10. https://doi.org/10.1093/jisesa/ieac017

Phiri BJ, Fèvre D, Hidano A. Uptrend in global managed honey bee colonies and production based on a six-decade viewpoint, 1961–2017. Sci Rep. **2022**:12(1):21298. https://doi.org/10.1038/s41598-022-25290-3

Potter NN, Hotchkiss JH. Food science. New York: Springer Science & Business Media;**1998**.

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE. Global pollinator declines: trends, impacts and drivers. Trends Ecol Evol. **2010**:25(6):345–353. https://doi.org/10.1016/j.tree.2010.01.007

R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; **2022**. https://www.R-project.org/

Raymann K, Moran NA. The role of the gut microbiome in health and disease of adult honey bee workers. Curr Opin Insect Sci. **2018**:26:97–104. https://doi.org/10.1016/j.cois.2018.02.012

Sammataro D, Weiss M. Comparison of productivity of colonies of honey bees, *Apis mellifera*, supplemented with sucrose or high fructose corn syrup. J Insect Sci. **2013**:13:19. https://doi.org/10.1673/031.013.1901

Scharlaken B, de Graaf DC, Goossens K, Brunain M, Peelman LJ, Jacobs FJ. Reference gene selection for insect expression studies using quantitative real-time PCR: the head of the honeybee, *Apis mellifera*, after a bacterial challenge. J Insect Sci. **2008**:8(33):1–10. https://doi.org/10.1673/031.008.3301

Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc. **2008**:3(6):1101–1108. https://doi.org/10.1038/nprot.2008.73

Seehuus S-C, Norberg K, Gimsa U, Krekling T, Amdam GV. Reproductive protein protects functionally sterile honey bee workers from oxidative stress. Proc Natl Acad Sci USA. **2006**:103(4):962–967. https://doi.org/10.1073/pnas.0502681103

Seeley TD, Visscher PK. Survival of honeybees in cold climates: the critical timing of colony growth and reproduction. Ecol Entomol. **1985**:10(1):81–88. https://doi.org/10.1111/j.1365-2311.1985.tb00537.x

Sesta G, Oddo L, Nisi F, Ricci L. Effects of artificial sugar feeding on sugar composition of royal jelly. Apiacta. **2006**:41:60–70.

Severson DW, Erickson EH Jr. Honey bee (Hymenoptera: Apidae) colony performance in relation to supplemental carbohydrates. J Econ Entomol. **1984**:77(6):1473–1478. https://doi.org/10.1093/jee/77.6.1473

Shinoda T, Itoyama K. Juvenile hormone acid methyltransferase: a key regulatory enzyme for insect metamorphosis. Proc Natl Acad Sci USA. **2003**:100(21):11986–11991. https://doi.org/10.1073/pnas.2134232100

Standifer LN, Moeller FE, Kauffeld NM, Herbert EW Jr, Shimanuki H. Supplemental feeding of honey bee colonies. United States Department of Agriculture Information Bulletin No. 413, 8. **1977**.

Taylor MA, Robertson AW, Biggs PJ, Richards KK, Jones DF, Parkar SG. The effect of carbohydrate sources: sucrose, invert sugar and components of mānuka honey, on core bacteria in the digestive tract of adult honey bees (*Apis mellifera*). PLoS One. **2019**:14(12):e0225845. https://doi.org/10.1371/journal.pone.0225845

Toth AL, Robinson GE. Worker nutrition and division of labour in honeybees. Anim Behav. **2005**:69(2):427–435. https://doi.org/10.1016/j.anbehav.2004.03.017

vanEngelsdorp D, Hayes J, Underwood RM, Pettis J. A survey of honey bee colony losses in the U.S., fall 2007 to spring 2008. PLoS One. **2008**:3(12):e4071. https://doi.org/10.1371/journal.pone.0004071

vanEngelsdorp D, Meixner MD. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. J Invertebr Pathol. **2010**:103(Suppl 1):S80–S95. https://doi.org/10.1016/j.jip.2009.06.011

Wheeler MM, Robinson GE. Diet-dependent gene expression in honey bees: honey vs. sucrose or high fructose corn syrup. Sci Rep. **2014**:4:5726. https://doi.org/10.1038/srep05726

White JW, Doner LW. Honey composition and properties. In: Beekeeping in the United States. Philadelphia (PA): U.S. Department of Agriculture; **1980**. p. 82–91.

Whitfield CW, Ben-Shahar Y, Brillet C, Leoncini I, Crauser D, Leconte Y, Rodriguez-Zas S, Robinson GE. Genomic dissection of behavioral maturation in the honey bee. Proc Natl Acad Sci USA. **2006**:103(44):16068–16075. https://doi.org/10.1073/pnas.0606909103

Zheng H, Perreau J, Powell JE, Han B, Zhang Z, Kwong WK, Tringe SG, Moran NA. Division of labor in honey bee gut microbiota for plant polysaccharide digestion. Proc Natl Acad Sci USA. **2019**:116(51):25909–25916. https://doi.org/10.1073/pnas.1916224116

Zheng H, Powell JE, Steele MI, Dietrich C, Moran NA. Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling. Proc Natl Acad Sci USA. **2017**:114(18):4775–4780. https://doi.org/10.1073/pnas.1701819114