



Special Collection: Insects as feed and fertilizer: Sustainable solutions for food waste and animal production practices

Evaluation of lauric acid enhancement of black soldier fly larvae from coconut

Delbert M. Gatlin III¹, Pedro L. Pucci Figueiredo De Carvalho¹, Casey Flint^{2,✉},
Chelsea Miranda³, Jeffery K. Tomberlin^{2,*✉}

¹Department of Ecology & Conservation Biology, Texas A&M University System, College Station, TX, USA, ²Department of Entomology, Texas A&M University System, College Station, TX, USA, ³Department of Biological Sciences, Howard Payne University, Brownwood, TX, USA *Corresponding author, mail: jeffery.tomberlin@ag.tamu.edu

Subject Editor: Yan-Qun Liu

Received on 5 March 2024; revised on 4 April 2024; accepted on 26 April 2024

The current study evaluated the potential enhancement of lauric acid (LA) in black soldier fly, *Hermetia illucens*, (L.) (Diptera: Stratiomyidae) larvae (BSFL), a source of this short-chain fatty acid which has antimicrobial and immunostimulatory properties. Replicate groups of BSFL were reared on either the coconut or Gainesville diet for 7 days. After the rearing period, BSFL were harvested, purged, dried, and subjected to proximate, fatty acid and amino acid compositions, and pepsin digestibility analyses. Results demonstrate changes in proximate composition. BSFL reared on the coconut had significantly ($P=0.002$) higher lipid content (47.3% vs. 25.2%) on a dry-matter basis. The LA concentration in BSFL produced on the coconut was 31% greater than those reared on Gainesville, resulting in almost 150% more LA. Furthermore, BSFL-fed coconut had reduced crude protein (29.7% of dry weight) and ash (3.7% of dry weight) relative to those fed Gainesville (43.4% and 7.5% for crude protein and ash, respectively) but higher pepsin digestibility (91.0% vs. 87.0%). The relative amounts of various amino acids in the 2 BSFL meals did not differ extensively, with statistically lower concentrations of only phenylalanine and tryptophan and higher concentrations of alanine, arginine, isoleucine, leucine, and serine in BSFL reared on coconut. Results demonstrate that the nutritional composition of BSFL can be manipulated, and an enhancement of LA concentrations of 150% was achieved with coconut, which has value for BSFL as a feed for various livestock, including aquaculture. Lower protein content is a tradeoff in terms of BSFL value as a feed additive.

Key words: bioeconomy, circular economy, sustainability

Introduction

The proliferation of various infectious diseases is often associated with intensive animal production practices, as seen in various types of aquaculture (Lieke et al. 2020). Not surprisingly, the death of fish and other aquatic species due to infectious diseases is a primary factor negatively impacting the economic viability of such livestock production in the United States, as well as other countries. Thus, preventing disease is a priority, but often is not realized and results in the need for treatment (i.e., added expense). In recent years, the utilization of chemotherapeutics to treat pathogens, especially antibiotics for bacterial pathogens, has fallen out of favor due to numerous problems, including high costs, limited efficacy, antibiotic resistance, and potential environmental issues. As such, the use of feed additives, which can not only inhibit pathogens but also enhance

the immune response and increase the resistance of various production animals to various pathogens, has considerable potential (e.g., Gatlin III and Yamamoto 2022).

Lauric acid (LA) has been identified as one such natural compound with potential benefits to livestock health. This fatty acid is found naturally in various plant oils being a major component of palm kernel oil and coconut oil. LA is also present in high concentrations in insect fats and oils (Hossain et al. 2023, Suryati et al. 2023), particularly those from black soldier fly *Hermetia illucens*, (L.) (Diptera: Stratiomyidae) larvae (BSFL). As a medium-chain fatty acid, LA has a very high absorption efficiency, being used as a direct energy source rather than being stored as hepatic fat in terrestrial animals (Benzertiha et al. 2020). In addition, due to its rapid oxidation and low deposition rate, dietary LA supplementation can prevent hepatic steatosis and decrease lipid deposition in the intraperitoneal

fat tissue of some aquatic animals (Li et al. 2016, Belghit et al. 2018). However, the most interesting characteristic of LA is its inhibitory activity against viruses, bacteria, and parasites, with the potential to improve gut health and enhance the immune status of animals (Lieberman et al. 2006, Zeitz et al. 2015, Ushakova et al. 2016). LA is known for its antimicrobial effects on gut microbiota, being particularly active against Gram-positive bacteria (Dierick et al. 2002, Skřivanová et al. 2005, Spranghers et al. 2017). Positive effects of dietary BSFL supplementation along with poultry byproduct meal were reported for barramundi (*Later calcarifer*) in regard to gut histomorphology, immunity, and resistance to the bacteria *Vibrio harveyi* (Chaklader et al. 2019). Rainbow trout (*Oncorhynchus mykiss*) fed a diet in which 5% of soybean meal was replaced with BSFL meal also showed a significantly higher cumulative survival than fish fed the control diet 10 days after being challenged with *Aeromonas salmonicida* (Cho et al. 2022). After 6 wk of feeding the BSFL diet to trout, they showed significantly higher expression of interleukin 1 β , interleukin 8, and immunoglobulin M genes compared to fish fed the control diet. These results were largely attributed to the presence of high levels of LA in diets containing BSFL meals. Therefore, LA derived from insects could provide an important added value when insects are used as a protein and/or lipid source in the diets of monogastric animals, including fish (Gasco et al. 2018).

BSFL meals have been researched more than other insect meals. Historically, initial efforts focused on the use of BSFL meal as feed for poultry (Hale 1973) and fish (Bondari and Sheppard 1981, 1987) in terms of growth and survivorship. More recent initiatives have provided increasingly granulated exploration of the effects of dietary BSFL on feed conversion (Abd El-Hack et al. 2020), meat quality (Cullere et al. 2016), and gastrointestinal health (Biasato et al. 2019, 2020) of animals including swine (Spranghers et al. 2018) and various fish species (Hossain et al. 2023). Given the fact that BSFL meal is a rich source of LA and has numerous potential health benefits, additional investigations with various animal species focusing specifically on health effects are anticipated. The objectives of the current study were to investigate the degree to which LA could be increased in BSFL using fruit naturally rich in LA. Examples include palm kernel, which was previously mentioned, as well as lemon, kumquat, and durian. In this study, coconut was used. This was accomplished by conducting 2 separate rearing trials and comprehensively evaluating the nutrient composition of BSFL reared on standard and coconut substrates.

Materials and Methods

Acquisition of BSFL

The BSFL were purchased from EVO Conversion Systems, LLC (College Station, TX, USA). The average initial larval weight was measured on an Adventurer PRO AV64 (Ohaus Cooperation, Pine Brook, NJ, USA). BSFL used in this experiment weighed approximately 0.011 g at the start of the experiment. BSFL were stored in a Rhee Environmental Chamber (Ashville, NC, USA) at 26 °C, 60% relative humidity, and 16L:8D until the initiation of the experiment.

Acquisition of BSFL and Diet Preparation

Larvae were fed either desiccated coconut (Celebes Coconut Cooperation, Barangay Banza, Butuan City, Philippines) or a control (Gainesville diet: 50% wheat bran, 30% alfalfa meal, 20% yellow corn flour) (Hogsette 1992) in 2 separate trials. Each coconut treatment consisted of 2.4 kg of desiccated coconut placed inside a Hefty 2.5-gal Jumbo Storage Slider Bag with 5.6 kg of reverse osmosis (RO) water. Twelve coconut treatment replicates were prepared due to the uncertainty of larval development to harvest.

Preliminary studies showed that desiccated coconut needed at least 24 h to absorb the water; therefore, the mixture was allowed to soak in the storage bag for 24 h before being used in the study. The dry Gainesville diet was hydrated to 70% moisture in a similar manner. The control diet was prepared by mixing approximately 2.4 kg of dry Gainesville diet with 5.6 kg of RO water immediately before use, as it did not need to be soaked. Three Gainesville control replicates were prepared.

Experiment Design

Each replicate was inoculated with approximately 6,000 14-day-old larvae fed a total of 8 kg of coconut or Gainesville diet (i.e., treatments) as described above. presoaked coconut or Gainesville diet was placed in the center of a 5.7-L Sterilite clear storage container (Townsend, MA, USA) with 800 g of dry Gainesville to prevent developing larvae from escaping. Larvae were placed on top of the diet in the center and allowed to feed for 7 days in the environmental chamber described above. To determine average larval weight at the time of harvest, 4 coconut replicates were randomly selected as a subsample, and all 3 Gainesville replicates were used to estimate the average final individual larval weight. Based on appearance, 10 largest larvae were selected from each replicate and weighed on the Adventurer PRO scale described above to determine the average final larval weight. This approach was taken to standardize daily measurements and minimize disrupting larval-substrate dynamics. The remaining larvae were separated from their diets via negative phototaxis, which included placing larvae and their diet on a 1/8" metal screen placed over a 5.7-L Sterilite clear storage container. Sunlight was used as the light source to drive larvae through the metal screen. Sifted larvae crawled through the screen and collected inside the Sterilite container. Collected larvae from the replicate containers associated with each treatment were pooled, placed in 3.8-L Ziploc Freezer Bags (S.C. Johnson & Son, Racine, WI, USA) and stored at -20 °C until dried. Aliquots of approximately 500 g of frozen BSFL biomass from each treatment were spread over metal trays (50 cm × 50 cm) and oven-dried for 36 h at 50 °C to achieve a constant weight. After cooling, the dried larvae associated with each treatment were pooled to provide 1 composite sample per treatment and were stored at -20 °C until analyzed for biochemical composition.

Biochemical Analysis

All nutritional analyses were conducted on duplicate samples of finely ground BSFL biomass reared on each substrate. The only exception was pepsin digestibility, for which only one determination was made by a commercial laboratory (SDK Laboratories, Hutchinson, KS, USA). The proximate composition of dried BSFL samples was measured in accordance with standard methods (AOAC 2005). Briefly, dry-matter content was determined gravimetrically by drying the ground samples overnight at 105 °C. Crude protein was estimated as $N \times 6.25$ by a LECO 828 Nitrogen and Protein Determinator (St. Joseph, MI, USA). Ash/organic matter content was determined by combustion in a furnace at 650 °C for 3 h, and crude lipid content was determined by chloroform-methanol extraction (Folch et al. 1957). A lipid droplet subsample was isolated and preserved in N_2 at -80 °C prior to the characterization of the fatty acid profile by flame-ionized gas chromatography (Smith et al. 2012). The monounsaturated/saturated fatty acid ratio (MUFA:SFA ratio) was calculated by dividing MUFA values by SFA values according to the formula: $(C16:1 + C18:1C + C18:1T)/(C12:0 + C14:0 + C16:0 + C18:0)$, where C16:1 is palmitoleate; C18:1c is oleate; C18:1t is vaccenate; C12:0 is laurate; C14:0 is myristate; C16:0 is

palmitate; and C18:0 is stearate. The quantification of amino acids in samples of BSFL reared on different substrates was accomplished using a UPLC system with an integrated tunable ultraviolet detector (Acquity system, Waters Corporation, Milford, MA, USA) and MassTrak AAA Solutions Kit (Waters Corporation, Milford, MA, USA). Samples were digested in 6 N HCl (BDH3028; VWR International, Center Valley, PA, USA) at 110 °C for 24 h, after which they were deproteinized with 1.5 mol/L HClO_4 (9552-05; JT Baker, Center Valley, PA, USA), neutralized with 2 mol/L K_2CO_3 (P5833; Sigma-Aldrich, St. Louis, MO, USA), and centrifuged (12,000 $\times g$, 5 min). The supernatant fluid was filtered through 0.2- μm polycarbonate syringe filters (28145-491; VWR International, Center Valley, PA, USA) before derivatization and analysis as previously described (Castillo et al. 2015).

Statistical Analysis

Data for percent dry matter, protein, lipid, percent ash, pepsin digestibility, and LA content were collected for dried BSFL produced from Gainesville or coconut substrates. Similar data were collected for all amino acids and fatty acids ranging from C12 to C20. For each variable, and following tests for normality (Kolmogorov-Smirnov) and equal variance (Bartlett's) being confirmed, a Student's *t*-test was utilized to establish significant differences between the 2 treatments. A *t*-test was used to determine differences in final larval weight, given that the data met basic assumptions. Differences were considered significant when the probability value was $P < 0.05$.

Results

BSFL in all replicates reared on both the control and coconut substrates appeared to grow and survive normally over the 7-day period. Larvae fed coconut were significantly ($P < 0.001$) larger (0.170 g \pm 0.066 g) than those fed Gainesville (0.148 g \pm 0.041 g). Analyses demonstrate differences in the proximate composition of harvested BSFL (Table 1). The BSFL reared on the coconut substrate had significantly ($P = 0.002$) higher lipid content but significantly reduced crude protein ($P = 0.001$) and ash ($P = 0.002$) composition compared to those fed the control substrate. The pepsin digestibility of BSFL reared on the coconut substrate was also numerically higher than that of the control substrate, but due to a lack of replicate observations, statistical analysis could not be performed (Table 1). Fatty acid analysis revealed that the LA concentration of BSFL produced on the coconut substrate was over 31% higher than those reared on the Gainesville substrate when expressed as a percentage of total fatty acids (Table 2). Myristic acid also was significantly elevated ($P = 0.001$) in BSFL from the coconut treatment, while significant reductions in palmitic acid ($P = 0.001$), oleic acid ($P = 0.001$), and linoleic acid ($P = 0.001$) were observed compared to BSFL reared on the control substrate. As such, larvae reared on the coconut substrate contained over 2.5 times more LA due to their higher lipid level

and more concentrated LA content (Fig. 1). The relative amounts of various dispensable and indispensable amino acids in the 2 BSFL meals were generally higher for BSFL reared on the control substrate compared to the coconut substrate (Table 3) with the following being significantly different across the 2 treatments. This was linked to the higher crude protein of BSFL from the control substrate. However, when expressed as a percentage of total amino acids, the composition of the 2 meals was more similar, with statistically higher levels of only phenylalanine ($P = 0.05$) and tryptophan ($P = 0.03$) in BSFL from the control substrate compared to the coconut substrate. Additionally, concentrations of alanine ($P = 0.007$), arginine ($P = 0.01$), isoleucine ($P = 0.01$), leucine ($P = 0.002$), and serine ($P = 0.008$) were higher in BSFL reared on coconut substrate (Table 3).

Discussion

Data from this study demonstrated LA can be readily enhanced in BSFL. In fact, data generated from the current study exceeded expectations with over a 150% increase in LA content. However, it should be noted that shifts in LA amount were not unexpected as previous work demonstrated different BSFL diets such as sewage sludge, fruit waste, and palm decanter resulted in LA concentrations in BSFL, expressed as a percentage of total fatty acids, of 58.3%, 76.1%, and 48.1%, respectively (Leong et al. 2015). Those 3 substrates differed considerably in moisture, crude protein, and lipid composition, with the fruit waste resulting in BSFL with the highest lipid content of 47.4% of dry weight, which is similar to the 47.1% lipid content of BSFL reared on the coconut substrate in the present study. However, considering the LA content of BSFL reared on fruit waste accounted for 76.1% of total fatty acids, that meal provided 36.1 g of LA/100 g of dry meal compared to 19.8 g LA/100 g from the BSFL reared on coconut substrate in the present study. Another study in which BSFL were reared on raw coconut endosperm waste reported a lipid content of 32% of dry weight with 55% of the fatty acids consisting of LA for a total contribution of 17.6 g LA/100 g of dry BSFL meal (Alifian et al. 2019), which is somewhat lower than achieved in the current study. The level of LA incorporated by BSFL reared on coconut in the present study is at the upper end of the range reported for various BSFL life stages as influenced by different rearing substrates when taking into account both the total lipid and LA concentrations of the BSFL (Suryati et al. 2023).

Yet, the relationship between dietary LA content (along with other nutritional constituents) and the degree of LA accumulation in larvae remains unclear. A few studies suggest that BSFL LA contents are not entirely dependent on the initial LA contents of the larval diet. For example, Ewald et al. (2020) fed 4 different larval diet substrates (i.e., bread, fish, food waste, and muscles) to BSFL and found up to 52% LA in BSFL-fed bread. Comparing this to the diet used in the current study, from a nutritional standpoint, on average, bread and coconut have different nutrient profiles, including

Table 1. Proximate composition and pepsin digestibility of BSFL (*Hermetia illucens*) reared in 2 different substrates. Values are means of 2 replicate analyses except for pepsin digestibility and are expressed in percentage (dry-matter basis)

Substrate	Dry matter %	Protein %	Lipid %	Ash %	Pepsin digestibility %
Gainesville	95.58 ^b	43.42 ^A	25.20 ^b	7.74 ^A	87.0
Coconut	97.45 ^A	29.74 ^b	47.31 ^A	3.68 ^b	91.0
<i>P</i> -value	0.001	0.000	0.002	0.002	–
PSE	0.28	0.95	3.73	0.64	–

PSE: pooled standard error. Different superscript letters in a line are significantly ($P < 0.05$) different according to Student's *t*-test.

Table 2. The fatty acid profile of BSFL, *Hermetia illucens*, reared in 2 different substrates. Values are means of duplicate determinations and expressed in percentage (dry-matter basis)

	Fatty acids	Gainesville (%)	Coconut (%)	P-value	PSD
C12:0	Laurate	31.90 ^b	41.84 ^a	0.003	1.91
C14:0	Myristate	10.73 ^b	22.95 ^a	0.000	1.27
C14:1 n-5	Myristoleate	0.75 ^b	0.83 ^a	0.043	0.03
C16:0	Palmitate	18.22 ^a	13.75 ^b	0.000	0.48
C16:1 n-7	Palmitoleate	3.73 ^b	4.02 ^a	0.020	0.09
C18:0	Stearate	4.02 ^a	3.34 ^b	0.033	0.26
C18:1 n-9	Oleate	15.78 ^a	11.05 ^b	0.000	0.53
C18:1 n-7	Vaccenate	0.98 ^a	0.00 ^b	0.000	0.03
C18:2 n-6	Linoleate	11.62 ^a	1.77 ^b	0.000	0.08
C18:3 n-3	α-Linolenate	1.12 ^a	0.23 ^b	0.000	0.01
C20:0	Arachidate	0.21 ^a	0.09 ^b	0.000	0.01
C20:1	11-Eicosenoate	0.40 ^a	0.03 ^b	0.004	0.07
C20:2	11-14-Eicosadienoate	0.06	0.00	0.158	0.03
C22:0	Behenate	0.06 ^a	0.00 ^b	0.001	0.01
C22:6 n-3	Docosahexaenoate	0.45	0.13	0.291	0.32
Total		100	100	—	—
MUFA:SFA ratio		0.35	0.20	—	—

PSE: pooled standard error. Different superscript letters in a line are significantly ($P < 0.05$) different according to Student's *t*-test. Bold indicates significant differences ($P < 0.05$).

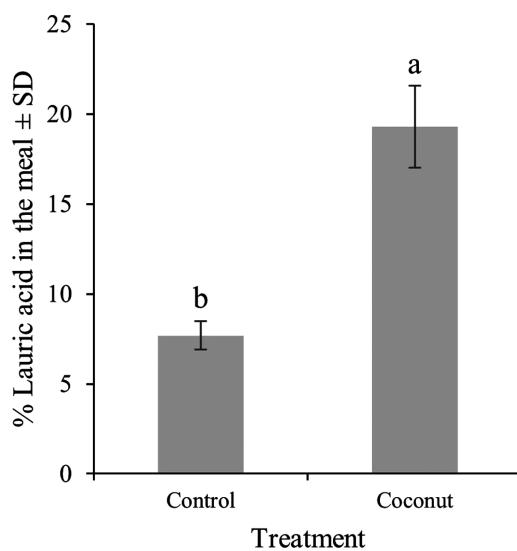


Fig. 1. The final concentration of lauric acid in meals obtained from BSFL (*Hermetia illucens*) reared on either the Gainesville diet or coconut. Values are expressed in g/100 g dry weight. A Student's *t*-test was used to compare lauric acid concentrations in BSFL reared on the Gainesville diet or coconut. Different lower-case letters indicate significant ($P < 0.05$) differences.

different LA contents (e.g., bread is typically low in saturated fatty acids). Yet, LA content is similar between the aforementioned study and the current study (52% compared to 42% of total fatty acids in the current study). [Ewald et al. \(2020\)](#) suggested that BSFL synthesize LA from carbohydrates because their initial diets had limited LA content (0%–1%). Such supposition aligns well with the findings from [Li et al. \(2022\)](#), which tested a soybean meal-based diet formulated with different fat sources (e.g., linseed oil, peanut oil, coconut oil, soybean oil, lard oil, and fish oil). Results showed that 19-day-old BSFL fed a diet formulated with 5%–10% coconut oil (with dietary LA comprising 38%–43% of the total fatty acid composition) had the highest larval LA content (38%–44%), which is also similar to our BSFL LA contents ([Li et al. 2022](#)). Interestingly,

BSFL-fed diets formulated with other oils whose initial LA content was below the detection limit (e.g., linseed, peanut, and soybean oil) yielded larvae with 22%–28% LA content. These oils typically do not contain any carbohydrates, but it is possible that BSF larvae synthesized LA from the carbohydrates in the soybean meal-based diet, which accounted for 90%–95% of the larval diet. Therefore, other nutritional constituents, like carbohydrates, in addition to dietary LA content, should be investigated to enhance LA in BSFL. With greater reason, efforts examining fine-scale nutritional variation beyond protein, fat, or carbohydrate content of substrates for growing BSFL are needed to optimize the production characteristics and nutritional value of the resulting BSFL meal.

The diet provided to BSFL clearly impacts their resulting nutritional profile. Numerous studies have demonstrated shifts in diet impact protein and carbohydrate values in resulting BSFL. Due to such interest in said research, at least 2 review articles have been published on this topic where shifts in protein (32%–58%) and fat (15%–39%) content ([Gold et al. 2018](#)) have been well-documented ([Surendra et al. 2020](#)). However, macronutrients are only one facet of feed production, and deeper dives into individual components are essential to determine potential bottlenecks that might be hidden if otherwise not investigated. While this is important for LA and other fatty acids, the same can be said for essential amino acids ([Tomberlin et al. 2023](#)).

LA may enhance the health of various mass-produced species. This may be attributed to the antimicrobial activity of the compound, which has been established in several animals (reviewed by [Borrelli et al. \(2021\)](#)). For example, [Rimoldi et al. \(2019\)](#) reported that fish gut health was improved by feeding BSFL to rainbow trout by influencing their intestinal bacterial communities. [Benzertiha et al. \(2020\)](#) also mentioned in their review article the positive effects of insect oils in stimulating the immune system of various animals. However, they also suggested that more research is needed to characterize potential modes of action and immune-related genes involved in fat metabolism more fully. It also should be noted that in addition to being a rich source of LA, BSFL also contains other constituents, such as chitin and antimicrobial peptides that are known to influence animal health ([Gasco et al. 2018](#)). Thus, investigations to

Table 3. The amino acid profile of BSFL, *Hermetia illucens*, reared in 2 different substrates. Values are means of duplicate determinations and expressed as g kg⁻¹ or percentage of total amino acids (dry-matter basis)

Amino acid	Gainesville	Coconut	PSE	Prob > F	Gainesville	Coconut	PSE	Prob > F
	(g kg ⁻¹)				(% of total amino acids)			
Ala	29.95 ^A	23.63 ^B	0.21	0.002	6.90 ^B	7.95 ^A	0.06	0.007
b-Ala	0.67 ^A	0.19 ^B	0.04	0.01	0.15 ^A	0.07 ^B	0.01	0.03
Arg	16.33	15.56	0.42	0.32	3.76 ^B	5.23 ^A	0.13	0.01
Asn	0.24	0.21	0.02	0.42	0.05	0.07	0.003	0.10
Asp	41.38 ^A	26.17 ^B	0.6	0.003	9.53	8.80	0.21	0.13
Cit	3.92	3.28	0.41	0.39	0.90	1.10	0.10	0.28
Cys	ND	ND	—	—	ND	ND	—	—
Glu	50.47 ^A	37.38 ^B	0.64	0.01	11.62	12.57	0.21	0.09
Gly	24.56 ^A	17.90 ^B	0.3	0.004	5.66	6.02	0.09	0.10
His	11.14 ^A	7.10 ^B	0.5	0.03	2.57	2.39	0.12	0.39
Ile	19.41 ^A	13.99 ^B	0.08	0.0004	4.47 ^B	4.71 ^A	0.02	0.01
Leu	30.35 ^A	22.19 ^B	0.27	0.002	6.99 ^B	7.46 ^A	0.09	0.06
Lys	22.38 ^A	13.95 ^B	0.64	0.01	5.15	4.69	0.17	0.20
Met	6.70	5.36	0.35	0.11	1.54	1.80	0.09	0.17
Orn	1.70 ^A	0.91 ^B	0.09	0.03	0.39	0.31	0.02	0.11
Phe	17.67 ^A	11.14 ^B	0.18	0.002	4.07 ^A	3.75 ^B	0.05	0.05
Pro	ND	ND	—	—	ND	ND	—	—
Ser	16.83 ^A	14.79 ^B	0.22	0.02	3.88 ^B	4.98 ^A	0.07	0.008
Tau	0.25	0.20	0.04	0.42	0.06	0.07	0.007	0.42
Thr	19.62	16.74	1.02	0.19	4.52	5.63	0.24	0.08
Trp	8.68 ^A	5.16 ^B	0.12	0.002	2.00 ^A	1.73 ^B	0.03	0.03
Tyr	25.08 ^A	16.75 ^B	0.16	0.0007	5.78	5.63	0.05	0.21
Val	29.50 ^A	21.31 ^B	0.27	0.002	6.79	7.17	0.07	0.06

PSE: standard pooled error; ND = not determined. Different superscript letters in a line are significantly ($P < 0.05$) different according to Student's *t*-test.

differentiate the effects of these various constituents in BSFL on animal health, as well as the influence of rearing substrate on these constituents, are warranted.

Unfortunately, organic side streams that can be used for mass-producing BSFL are limited due to regulations. Currently, in the United States, the Association of American Feed Control Officials (AAFCO) has only recommended and approved preconsumer food waste as a substrate to be provided to BSFL, which is then used as a feed ingredient. Such a conservative regulation for opening channels of organic side streams (e.g., manure, postconsumer food waste) for use in such processes is understandable considering concerns with pathogens, contaminants (e.g., heavy metals), and toxins. However, such an approach restricts the ability of the industry to stabilize, expand, and truly offer resources at a level that allows for their use as a feed. Thus, lines of communication between regulatory bodies, such as AAFCO, Federal Drug Administration, United States Department of Agriculture Agricultural Research Service (USDA-ARS), trade organizations (e.g., North American Coalition for Insect Agriculture), and researchers (e.g., National Science Foundation Center for Environmental Sustainability through Insect Farming (CEIF)) are needed. Establishing such lines of communication could result in a more efficient expansion of resources available for BSFL production, which in turn removes impediments, allowing for the growth of this new sector of agriculture.

Optimizing feed for use in the aquaculture industry is critical for various economic and environmental reasons. Besides producing fish and other aquatic species at minimal expense to increase profitability and availability to more people, doing so also reduces the impact of the industry on the environment. For example, using BSFL meal may reduce the need for fishmeal, which in turn reduces the burden on international fisheries concerned with harvesting

beyond sustainable levels. One aspect of optimizing feed is creating ingredients that may enhance the health of the animal consuming it. As in the case of LA, enhanced levels of BSFL for incorporation into aquaculture feed may result in various benefits, including decreased susceptibility to pathogens and other diseases. However, this work is only the beginning as efforts are needed to determine if the increased LA in combination with the other nutrients associated with BSFL meal synergize with one another, resulting in the benefits previously mentioned.

Funding

This work was supported by the National Science Foundation through the Industry-University Cooperative Research Centers (IUCRC) NSF cooperative agreements NSF-IIP-2052454 (TAMU), NSF-IIP-2052565 (IUI), and NSF-IIP-2052788 (MSU). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation or the Industry Advisory Board Members of the Center for Environmental Sustainability through Insect Farming. The authors appreciate financial support from the Texas A&M AgriLife Research State Air Quality Initiative (SAQI).

Conflict of Interest

Material for use in this project was purchased from EVO Conversion Systems LLC, a company with which Dr. Tomberlin has a significant financial interest. This conflict of interest is managed by a plan submitted to and approved by Texas A&M University and Texas A&M AgriLife.

Author Contributions

Delbert Gatlin (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Funding acquisition [equal], Investigation [equal], Methodology [equal], Project administration [equal], Resources [equal], Supervision [equal], Writing—original draft [equal], Writing—review & editing [equal]), Pedro Pucci Figueiredo De Carvalho (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Investigation [equal], Methodology [equal], Project administration [equal], Visualization [equal], Writing—original draft [equal], Writing—review & editing [equal]), Casey Flint (Conceptualization [equal], Data curation [equal], Methodology [equal], Project administration [equal], Writing—review & editing [equal]), Chelsea Miranda (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Investigation [equal], Methodology [equal], Project administration [equal], Writing—original draft [equal], Writing—review & editing [equal]), and Jeffery Tomberlin (Conceptualization [equal], Funding acquisition [equal], Investigation [equal], Methodology [equal], Project administration [equal], Resources [equal], Writing—original draft [equal], Writing—review & editing [equal])

References

Abd El-Hack ME, Shafi ME, Alghamdi WY, Abdelnour SA, Shehata AM, Noreldin AE, Ashour EA, Swelum AA, Al-Sagan AA, Alkhateeb M, et al. Black soldier fly (*Hermetia illucens*) meal as a promising feed ingredient for poultry: a comprehensive review. *Agriculture*. 2020;10(8):339.

Alifan M, Sholikin M, Evyernie D, Nahrowi. Potential fatty acid composition of *Hermetia illucens* oil reared on different substrates. In: IOP Conference Series: Materials Science and Engineering. Malang, Indonesia: IOP Publishing; 2019. p. 062002.

AOAC (Association of Official Analytical Chemists). Official methods of analysis. Washington (DC): AOAC; 2005.

Belghit I, Liland NS, Gjesdal P, Biancarosa I, Menchetti E, Li Y, Waagbø R, Krogdahl A, Lock E-J. Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (*Salmo salar*). *Aquaculture*. 2018;503:609–619. <https://doi.org/10.1016/j.aquaculture.2018.12.032>

Benzertiha A, Kierofczyk B, Rawski Mikolajczak MZ, Urbański A, Nogowski L, Józefiak D. Insect fat in animal nutrition—a review. *Ann Anim Sci*. 2020;20(4):1217–1240.

Biasato I, Ferrocino I, Dabbou S, Evangelista R, Gai F, Gasco L, Cocolin L, Capucchio MT, Schiavone A. Black soldier fly and gut health in broiler chickens: insights into the relationship between cecal microbiota and intestinal mucin composition. *J Anim Sci Biotechnol*. 2020;11:11. <https://doi.org/10.1186/s40104-019-0413-y>

Biasato I, Renna M, Gai F, Dabbou S, Meneguz SM, Perona G, Martinez S, Lajusticia ACB, Bergagna Sardi SL, Capucchio MT, et al. Partially defatted black soldier fly larva meal inclusion in piglet diets: effects on the growth performance, nutrient digestibility, blood profile, gut morphology and histological features. *J Anim Sci Biotechnol*. 2019;10:12–12.

Bondari K, Sheppard DC. Soldier fly larvae as feed in commercial fish production. *Aquaculture*. 1981;24:103–109. [https://doi.org/10.1016/0044-8486\(81\)90047-8](https://doi.org/10.1016/0044-8486(81)90047-8)

Bondari K, Sheppard DC. Soldier fly, *Hermetia illucens* L., larvae as feed for channel catfish, *Ictalurus punctatus* (Rafinesque), and blue tilapia, *Oreochromis aureus* (Steindachner). *Aquacult Res*. 1987;18(3):209–220. <https://doi.org/10.1111/j.1365-2109.1987.tb00141.x>

Borrelli L, Varriale L, Dipineto L, Pace A, Menna LF, Fioretti A. Insect derived lauric acid as promising alternative strategy to antibiotics in the antimicrobial resistance scenario. *Front Microbiol*. 2021;12:620798. <https://doi.org/10.3389/fmicb.2021.620798>

Castillo S, Halligan S, Gatlin DM III. Growth responses of juvenile red drum *Sciaenops ocellatus* to dietary phenylalanine and tyrosine can be used to calculate the total aromatic amino acid requirement. *J Nutr*. 2015;145(10):2341–2346. <https://doi.org/10.3945/jn.115.215848>

Chaklader MR, Siddik MA, Fotedar R, Howieson J. Insect larvae, *Hermetia illucens* in poultry by-product meal for barramundi, *Lates calcarifer* modulates histomorphology, immunity and resistance to *Vibrio harveyi*. *Sci Rep*. 2019;9:16703.

Cho J-H, Bae J, Hwang IJ. Effects of black soldier fly (*Hermetia illucens*) pre-pupae meal on the growth, stress, and immune responses of juvenile rainbow trout (*Oncorhynchus mykiss*) reared at different stocking densities. *Aquacult Rep*. 2022;25:101202. <https://doi.org/10.1016/j.aqrep.2022.101202>

Cullere M, Tasoniero G, Giaccone V, Miotti-Scapin R, Claeys E, De Smet S, Dalle Zotte A. Black soldier fly as dietary protein source for broiler quails: apparent digestibility, excreta microbial load, feed choice, performance, carcass and meat traits. *Animal*. 2016;10(12):1923–1930. <https://doi.org/10.1017/S1751731116001270>

Dierick N, Decuyper J, Molly K, Van Beek E, Vanderbeke E. The combined use of triacylglycerols (TAGs) containing medium chain fatty acids (MCFAs) and exogenous lipolytic enzymes as an alternative to nutritional antibiotics in piglet nutrition: II. *In vivo* release of MCFAs in gastric cannulated and slaughtered piglets by endogenous and exogenous lipases; effects on the luminal gut flora and growth performance. *Livest Prod Sci*. 2002;76(1–2):1–16. [https://doi.org/10.1016/s0301-6226\(01\)00331-1](https://doi.org/10.1016/s0301-6226(01)00331-1)

Ewald N, Vidakovic A, Langeland M, Kiessling A, Sampels S, Lalander C. Fatty acid composition of black soldier fly larvae (*Hermetia illucens*)—possibilities and limitations for modification through diet. *Waste Manag*. 2020;102:40–47. <https://doi.org/10.1016/j.wasman.2019.10.014>

Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem*. 1957;226(1):497–509.

Gasco L, Finke M, Van Huis A. Can diets containing insects promote animal health? *J Insects Food Feed*. 2018;4(1):1–4. <https://doi.org/10.3920/jiff2018.x001>

Gatlin DM III, Yamamoto FY. Nutritional supplements and fish health. In: Hardy R, editor. *Fish nutrition*. Amsterdam, The Netherlands: Elsevier; 2022. p. 745–773.

Gold M, Tomberlin JK, Diener S, Zurbrügg C, Mathys A. Decomposition of biowaste macronutrients, microbes, and chemicals in black soldier fly larval treatment: a review. *Waste Manag*. 2018;82:302–318. <https://doi.org/10.1016/j.wasman.2018.10.022>

Hale OM. Dried *Hermetia illucens* larvae (Stratiomyidae) as a feed additive for poultry. *J Ga Entomol Soc*. 1973;8:16–20.

Hogsette JA. New diets for production of house flies and stable flies (Diptera: Muscidae) in the laboratory. *J Econ Entomol*. 1992;85(6):2291–2294. <https://doi.org/10.1093/jee/85.6.2291>

Hossain MS, Small BC, Hardy R. Insect lipid in fish nutrition: recent knowledge and future application in aquaculture. *Rev Aquacult*. 2023;15(4):1664–1685. <https://doi.org/10.1111/raq.12810>

Leong S, Kutty S, Tan C, Tey L. Comparative study on the effect of organic waste on lauric acid produced by *Hermetia illucens* larvae via bioconversion. *J Eng Sci Technol*. 2015;8:52–63.

Li S, Ji H, Zhang B, Tian J, Zhou J, Yu H. Influence of black soldier fly (*Hermetia illucens*) larvae oil on growth performance, body composition, tissue fatty acid composition and lipid deposition in juvenile Jian carp (*Cyprinus carpio* var. Jian). *Aquaculture*. 2016;465:43–52. <https://doi.org/10.1016/j.aquaculture.2016.08.020>

Li X, Dong Y, Sun Q, Tan X, You C, Huang Y, Zhou M M. Growth and fatty acid composition of black soldier fly *Hermetia illucens* (Diptera: Stratiomyidae) larvae are influenced by dietary fat sources and levels. *Animals*. 2022;12(4):486.

Lieberman S, Enig MG, Preuss HG. A review of monolaurin and lauric acid: natural virucidal and bactericidal agents. *Alter Complement Ther*. 2006;12(6):310–314.

Liecke T, Meimelt T, Hoseinifar SH, Pan B, Straus DL, Steinberg CE. Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases. *Rev Aquacult*. 2020;12:943–965.

Rimoldi S, Gini E, Iannini F, Gasco L, Terova G. The effects of dietary insect meal from *Hermetia illucens* prepupae on autochthonous gut microbiota of rainbow trout (*Oncorhynchus mykiss*). *Animals*. 2019;9(4):143. <https://doi.org/10.3390/ani9040143>

Skřivanová E, Marounek M, Dlouha G, Kaňka J. Susceptibility of *Clostridium perfringens* to C2–C18 fatty acids. *Lett Appl Microbiol.* 2005;41(1):77–81.

Smith S, Go G, Johnson B, Chung K, Choi S, Sawyer J, Silvey D, Gilmore L, Ghahramany G, Kim K. Adipogenic gene expression and fatty acid composition in subcutaneous adipose tissue depots of Angus steers between 9 and 16 months of age. *J Anim Sci.* 2012;90(8):2505–2514.

Spranghers T, Michiels J, Vrancx J, Ovyn A, Eeckhout M, De Clercq P, De Smet S. Gut antimicrobial effects and nutritional value of black soldier fly (*Hermetia illucens* L.) prepupae for weaned piglets. *Anim Feed Sci Technol.* 2018;235:33–42.

Spranghers T, Ottoboni M, Klootwijk C, Ovyn A, Deboosere S, De Meulenaer B, Michiels J, Eeckhout M, De Clercq P, De Smet S. Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. *J Sci Food Agric.* 2017;97(8):2594–2600. <https://doi.org/10.1002/jsfa.8081>

Surendra KC, Tomberlin JK, van Huis A, Cammack JA, Heckmann L-HL, Khanal SK. Rethinking organic wastes bioconversion: evaluating the potential of the black soldier fly (*Hermetia illucens* (L.)) (Diptera: Stratiomyidae) (BSF). *Waste Manag.* 2020;117(117):58–80. <https://doi.org/10.1016/j.wasman.2020.07.050>

Suryati T, Julaeha E E, Farabi K, Ambarsari H, Hidayat AT. Lauric acid from the black soldier fly (*Hermetia illucens*) and its potential applications. *Sustainability.* 2023;15:10383.

Tomberlin JK, Miranda C, Flint C, Harris E, Wu G. Nutrients limit production of insects for food and feed: an emphasis on nutritionally essential amino acids. *Animal Front.* 2023;13(4):64–71. <https://doi.org/10.1093/af/vfad032>

Ushakova NA, Brodskii ES, Kovalenko AA, Bastrakov AI, Kozlova AA, Pavlov DS. Characteristics of lipid fractions of larvae of the black soldier fly *Hermetia illucens*. *Dokl Biochem Biophys.* 2016;468(1):209–212. <https://doi.org/10.1134/S1607672916030145>

Zeitz J, Fennhoff J, Kluge H, Stangl G, Eder K. Effects of dietary fats rich in lauric and myristic acid on performance, intestinal morphology, gut microbes, and meat quality in broilers. *Poult Sci.* 2015;94(10):2404–2413. <https://doi.org/10.3382/ps/pev191>