AMERICAN SOCIETY OF ANIMAL SCIENCE

Journal of Animal Science, 2021, Vol. 99, No. 6, 1–10

doi:10.1093/jas/skab124
Advance Access publication April 21, 2021
Received: 2 February 2021 and **Accepted:** 15 April 2021
Companion Animal Nutrition

COMPANION ANIMAL NUTRITION

Amino acid digestibility and digestible indispensable amino acid score-like values of black soldier fly larvae fed different forms and concentrations of calcium using the precision-fed cecectomized rooster assay

Sungho Do,[†] Elizabeth A. Koutsos,[‡] Pamela L. Utterback,[†] Carl M. Parsons,[†] Maria R. C. de Godoy,[†,‖] and Kelly S. Swanson[†,‖,$,1]

[†]Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA, [‡]EnviroFlight, Yellow Springs, OH 45387, USA, [‖]Division of Nutritional Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA, [$]Department of Veterinary Clinical Medicine, University of Illinois at Urbana-Champaign, Urbana IL 61801, USA

[1]Corresponding author: ksswanso@illinois.edu

ORCiD number: 0000-0001-5518-3076 (K. S. Swanson).

Abstract

Black soldier fly larvae (**BSFL**) are an alternative protein source for animals, including dogs and cats. Dietary calcium source is an essential nutrient for BSFL development in the pupal stage. Calcium carbonate ($CaCO_3$) and calcium chloride ($CaCl_2$) are common calcium sources but differ in solubility, acid-binding capacity, and calcium concentration. A high calcium concentration in BSFL may affect how well nitrogen and amino acids (**AA**) are digested by animals consuming them, thereby affecting feed conversion efficiency. Our objective was to determine the effects of dietary calcium form and concentration on nutrient composition, AA digestibility, and digestible indispensable amino acid score (**DIAAS**)-like values of BSFL intended for use in animal feeds using the precision-fed cecectomized rooster assay. All BSFL tested in this study were harvested at 18 d after hatch. Industry standard rearing conditions were maintained and a commercial layer ration was fed to all BSFL until 11 d post-hatch. From day 11 to 18, BSFL were fed a combination of distiller's dried grains with solubles from a distillery, bakery byproduct meal, and varied calcium sources. All BSFL diets contained 0.2% calcium in the basal diet plus additional calcium in the following amounts and forms: BSFLA: 1.2% $CaCl_2$, BSFLB: 1.2% $CaCO_3$, BSFLC: 0.75% $CaCO_3$, and BSFLD: 0.6% $CaCO_3$ + 0.6% $CaCl_2$. On day 18, BSFL were washed and frozen. Prior to the rooster assay, BSFL were lyophilized and ground. In total, 16 cecectomized roosters (4 roosters per substrate) were randomly assigned to test substrates. After 24 h of feed withdrawal, roosters were tube-fed 20 g of test substrates. Following crop intubation, excreta were collected for 48 h. Endogenous corrections for AA were made using five additional cecectomized roosters. All data were analyzed using a completely randomized design and the GLM procedure of SAS 9.4. Nutrient and AA digestibilities were not different among substrates. DIAAS-like values were calculated to determine protein quality according to the Association of American Feed Control Officials nutrient profiles and National Research Council recommended allowances for dogs and cats. Although AA digestibilities did not differ, those containing $CaCO_3$ generally had higher DIAAS-like reference values than the diet containing $CaCl_2$ alone (BSFLA). Aromatic AA (Phe + Tyr) and sulfur AA (Met + Cys) were often first-limiting AA. Our results suggest that calcium sources fed to BSFL did not affect AA digestibility and protein quality.

Key words: amino acid digestion, canine nutrition, feline nutrition, insect protein, pet food

Abbreviations

AA	amino acids
AAFCO	Association of American Feed Control Officials
ADF	acid detergent fiber
AHF	acid-hydrolyzed fat
BSFL	black soldier fly larvae
CP	crude protein
DM	dry matter
FAO	Food and Agriculture Organization
GE	gross energy
NDF	neutral detergent fiber
NRC	National Research Council
OM	organic matter

Introduction

Black soldier fly larvae (**BSFL**; *Hermetia illucens*) have been shown to efficiently convert various organic materials such as livestock manure, fruits, and vegetable waste into alternative protein and fat sources for swine (Newton et al., 1977, 2005), poultry (Cullere et al., 2016; Marono et al., 2017; Mwaniki et al., 2018), and fish species (Kroeckel et al., 2012; Lock et al., 2015). The environmental factors and housing conditions (e.g., temperature, humidity, and stocking density) used and dietary ingredient and nutrient composition fed to BSFL require consideration because they affect their growth rate, survival rate, and nutrient composition (Sheppard et al., 2002; Newton et al., 2005; Diener et al., 2009; Tomberlin et al., 2009; Holmes et al., 2012).

Chia et al. (2018), who tested nine temperatures (10 to 42 °C), demonstrated that housing temperature affected BSFL survival rate. Egg eclosion rate was highest at 30 °C (80%) and 35 °C (75%) and lowest at 15, 37, and 40 °C (below 11%). The survival rate of the larval and pupal stages was also highest at 30 °C (90% and 77%) and 35 °C (92% and 75%). Like temperature, relative humidity is also an essential factor affecting BSFL development and survival. Egg eclosion time (124.43 to 87.63 h) and mortality rate (62% to 3%) of the BSFL pupal stage decreased when the relative humidity increased from 25% to 70%. Also, adult longevity (5.17 to 7.94 d) and the emergence rate of BSF (16% to 93%) increased when they were reared on 40% and 70% of relative humidity compared with 25% (Holmes et al., 2012). Another aspect under consideration is stocking density. Barragan-Fonseca et al. (2018) placed 50, 100, 200, or 400 BSFL in plastic containers (15.5 × 10.5 × 6 cm), demonstrating that development time was shorter (13 vs. 15 d) at lower larval densities (50, 100, and 200) when raised on a diet with high nutrient concentrations (14.0% protein and 1.8% fat vs. 3.5% protein and 0.7% fat).

Dietary factors not only influence BSFL performance but also their nutrient composition. Oonincx et al. (2015) observed that when BSFL were fed a chicken feed diet (control diet), they had a shorter development time (20 d) than larvae fed animal manures (chicken: 144 d, pig: 144 d, and cow: 214 d). According to Nguyen et al. (2015), protein and fat concentrations of BSFL fed rendered fish (protein: 50% and fat: 36.2%) or pig liver (protein: 76.7% and fat: 12.8%) were higher than BSFL fed chicken feed (protein: 18% and fat: 2.52%). However, Bosch et al. (2014) reported that BSFL fed a broiler starter diet had protein (56.1%) and fat (12.8%) concentrations that were much higher than those fed a similar diet in the Nguyen et al. (2015) study. The mineral content of BSFL also depends on the diet fed. For instance, phosphorus and calcium concentrations of BSFL reared on poultry manure (1.5% and 7.8%) were higher than BSFL reared on swine manure (0.88% and 5.36%) or chicken feed (1.28% and 3.14%) (Newton et al., 2005; Dierenfeld and King, 2008; Finke, 2013).

BSFL contain high calcium concentrations and greater calcium, magnesium, and potassium concentrations than other insects, such as tebo worms, Turkestan cockroaches, and house flies (Finke, 2013). The reason that BSFL contain high calcium content is because their exoskeleton layer is rich in $CaCO_3$ (Johannsen, 1922). Dietary calcium sources for BSFL may impact the development of their epidermis in the prepupal to pupal stages. There are many dietary calcium options when it comes to feeding BSFL, with variance in acid-binding capacity, water solubility, and calcium concentration. To our knowledge, no scientific research has been conducted to test how dietary calcium form and concentration affect the growth rate of BSFL and their protein quality for use in animal feed. For this reason, our objective was to determine the effects of dietary calcium form and concentration on nutrient composition, amino acid (**AA**) digestibility, and digestible indispensable amino acid score (**DIAAS**)-like values of BSFL intended for use in animal feeds using the precision-fed cecectomized rooster assay. We hypothesized that the different forms and concentrations of dietary calcium sources used in this study would affect the nutrient composition of BSFL. However, we hypothesized that the protein quality of BSFL would not be changed.

Materials and Methods

Substrates

All BSFL tested in this study were harvested at 18 d after hatch. Industry standard rearing conditions were maintained (Sheppard et al., 2002) and a commercial layer ration was fed to all BSFL until 11 d post-hatch. From day 11 to 18, they were fed a combination of distiller's dried grains with solubles from a distillery, bakery byproduct meal, and calcium sources ($CaCl_2$ and $CaCO_3$). Treatment groups included the following:

1. **BSFLA** (0.2% calcium in basal diet + 1.2% Ca from $CaCl_2$)
2. **BSFLB** (0.2% calcium in basal diet + 1.2% Ca from $CaCO_3$)
3. **BSFLC** (0.2% calcium in basal diet + 0.75% Ca from $CaCO_3$)
4. **BSFLD** (0.2% calcium in basal diet + 0.6% Ca from $CaCO_3$ + 0.6 % Ca from $CaCl_2$)

Although $CaCl_2$ is the typical calcium source used to raise BSFL in production, it is expensive, so a more economical option was tested at various inclusion levels. Because calcium sources differ in solubility, calcium content, and acid-binding capacities, numerous options exist. In this initial study, it was of interest to test whether 50% or total replacement of the 1.2% $CaCl_2$ was possible without detrimental effects. On day 18, larvae were washed and frozen. All BSFL were then lyophilized and ground through a 2-mm screen with dry ice prior to chemical analysis and feeding to cecectomized roosters.

Cecectomized rooster assay

The protocol for the cecectomized rooster assay, including all animal housing, handling, and surgical procedures, was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign prior to experimentation. A precision-fed rooster assay using cecectomized Single Comb White Leghorn roosters was conducted as described by Parsons (1985) to determine the dry matter (**DM**), organic matter (**OM**), acid-hydrolyzed fat (**AHF**), and AA digestibility of the substrates listed above. Prior to the study, the cecectomy surgery was performed on roosters under general anesthesia according to the procedures of Parsons (1985).

Briefly, 16 cecectomized roosters were randomly assigned to the test substrates (4 roosters per test substrate evaluated). After 24 h of feed withdrawal, roosters were tube-fed 20 g of test substrates. Following crop intubation, excreta (urine and feces) were collected for 48 h on plastic trays placed under each individual cage. Excreta samples then were lyophilized, weighed, and ground through a 0.25-mm screen prior to analysis. Endogenous corrections for AA were made using five additional cecectomized roosters that had been fasted for 48 h. Nutrient and AA digestibilities were calculated using the method described by Sibbald (1979).

Chemical analyses

The substrates and rooster excreta were analyzed for DM (105 °C) and ash (OM was calculated based on ash) according to AOAC (2006; DM: method 934.01; OM: method 942.05). Nitrogen and crude protein (**CP**) were determined using a Leco Nitrogen/Protein Determinator (Model FP-2000, Leco Corporation, St. Joseph, MI) according to AOAC (2006; method 982.30E). Fat concentrations were measured by acid hydrolysis according to AACC (1983) followed by diethyl ether extraction (Budde, 1952). Gross energy (**GE**) was measured using a bomb calorimeter (Model 1261; Parr Instrument Co., Moline, IL). AA and calcium were measured at the University of Missouri Experiment Station Chemical Laboratories (Columbia, MO) according to the AOAC (2006) method 982.30 E [a, b, c] and method 968.08.

DIAAS-like calculations

The calculation of DIAAS-like values was followed according to Mathai et al. (2017) and Oba et al. (2019). The digestible indispensable AA reference ratios were calculated for each ingredient using the following equation (FAO, 2011): Digestible indispensable AA reference ratio = digestible indispensable AA content in 1 g protein of food (mg)/mg of the same dietary indispensable AA in 1 g of the reference protein.

The references used were the Association of American Feed Control Officials (AAFCO, 2019) nutrient profiles for adults at maintenance (dogs and cats) and growth and reproduction (dogs and cats) and National Research Council (NRC, 2006) recommended allowances for adults (dogs and cats), growing puppies (4 to 14 wk of age), and growing kittens.

The DIAAS-like values were then calculated using the following equation adapted from the Food and Agriculture Organization (FAO, 2011): DIAAS-like % = 100 × [(mg of digestible dietary indispensable AA in 1 g of the dietary protein)/(mg of the minimum recommendation of the same dietary indispensable AA in 1 g of the minimum protein recommendation)].

Statistical analysis

All rooster data were analyzed as a completely randomized design using the GLM procedure of Statistical Analysis Systems 9.4 (SAS Inst., Cary, NC). Substrates were considered to be a fixed effect. Tukey's multiple comparison analysis was used to compare least squares means and control for experiment-wise error. Differences were considered significant with $P < 0.05$.

Results

Chemical composition

The chemical composition of the tested BSFL is presented in Table 1. The chemical composition of all four BSFL treatments was similar, containing 87.8% to 89.5% OM, 40.0% to 41.7% CP, 31.1% to 33.5% AHF, and 2.87% to 3.47% calcium, and GE content between 5.61 and 5.85 kcal/g. Concentrations of indispensable and dispensable AA are presented in Table 2. AA concentrations and patterns of all BSFL were similar.

Cecectomized rooster assay

The digestibilities of DM, OM, and AHF were not different among BSFL tested (Table 1). There were no differences in indispensable and dispensable AA digestibilities for all BSFL tested (Table 3). All indispensable AA digestibilities were higher than 90%, with the exception of valine (81.29% to 84.03%). For most of the dispensable AA, digestibilities were greater than 90%. The exceptions were for cysteine (76.88% to 81.95%), glycine (79.1% to 86.38%), and serine (87.92% to 91.18%).

DIAAS-like calculations

DIAAS-like reference values for growing puppies and kittens are presented in Table 4 and Table 5, respectively. DIAAS-like reference values for adult dogs and cats at maintenance are presented in Table 6 and Table 7, respectively. With the exception of threonine and sulfur AA (methionine + cysteine), most of the BSFL tested had DIAAS-like values over 100%. For BSFLA, DIAAS-like reference values for arginine, sulfur AA (methionine + cysteine), and threonine were less than 100% when using the AAFCO recommended allowances for growing puppies. Based on the NRC recommended allowances for growing puppies, all BSFL had DIAAS-like reference values above 100%, with the exception of threonine and sulfur AA (methionine + cysteine). According to the AAFCO and NRC recommended allowances for growing kittens, all BSFL had DIAAS-like reference values above 100% with the exception of sulfur AA (methionine + cysteine) and aromatic AA (phenylalanine + tyrosine).

All BSFL ingredients had DIAAS-like reference values over 100%, with the exception of methionine and sulfur AA (methionine + cysteine), when using the AAFCO recommended allowances for adult dogs. Based on the NRC recommended allowances for an adult dog at maintenance, however, DIAAS-like reference values were lower than 100% for leucine, sulfur AA (methionine + cysteine), aromatic AA (phenylalanine + tyrosine), threonine, and tryptophan for most of the BSFL tested. According to the AAFCO and NRC recommended allowances for adult cats, all BSFL tested had DIAAS-like reference values over 100% with the exception of sulfur AA (methionine + cysteine) and aromatic AA (phenylalanine + tyrosine).

The first-limiting AA based on DIAAS-like reference values from AAFCO (2019) nutrient profiles for dogs and cats (growth and reproduction; adults at maintenance) are provided in Table 8. Sulfur AA (methionine + cysteine) was the first-limiting AA for all BSFL when calculating DIAAS-like reference values using AAFCO (2019) nutrient profiles for growing and reproducing dogs. Sulfur AA (methionine + cysteine) was also the first-limiting AA for all BSFL when calculating DIAAS-like reference values using AAFCO (2019) nutrient profiles for adult dogs and cats at maintenance. For these categories, all of the DIAAS-like reference values for limiting AA were less than 100, suggesting insufficiency if a diet was formulated with only that protein source and at an inclusion level to meet the nutrient profile. Sulfur AA (methionine + cysteine) was the first-limiting AA when calculating DIAAS-like reference values using the AAFCO (2019) nutrient profiles for adult cats at maintenance, with all values being greater than 100, suggesting sufficiency.

The first-limiting AA based on DIAAS-like reference values from NRC (2006) recommended allowances for growing puppies

Table 1. Chemical composition and nutrient digestibility of BSFL fed different forms and concentrations of calcium using the precision-fed cecectomized rooster assay

Item	BSFLA[1]	BSFLB[2]	BSFLC[3]	BSFLD[4]	SEM	P-value
Chemical composition						
DM, %	93.34	94.09	93.16	93.56	—	—
OM, % DM	88.07	88.06	89.49	87.84	—	—
CP, % DM	41.68	40.15	41.58	41.04	—	—
AHF, % DM	31.15	33.45	33.05	32.73	—	—
GE, kcal/g DM	5.63	5.84	5.85	5.61	—	—
Ca, % DM	3.20	3.42	2.87	3.47	—	—
Nutrient digestibility						
DM, %	62.30	60.39	62.55	59.77	1.403	0.8926
OM, %	74.19	71.02	74.19	68.63	1.173	0.3548
AHF, %	85.30	82.94	85.91	83.21	0.929	0.6373

[1] BSFLA: 0.2% calcium in basal diet + 1.2% $CaCl_2$.
[2] BSFLB: 0.2% calcium in basal + 1.2% $CaCO_3$.
[3] BSFLC: 0.2% calcium in basal diet + 0.75% $CaCO_3$.
[4] BSFLD: 0.2% calcium in basal diet + 0.6% $CaCO_3$ + 0.6% $CaCl_2$.

Table 2. Indispensable and dispensable AA concentrations (% DM) of BSFL fed different forms and concentrations of calcium

Item	BSFLA[1]	BSFLB[2]	BSFLC[3]	BSFLD[4]
Indispensable AA				
Arginine	1.92	1.99	2.02	2.00
Histidine	1.24	1.27	1.31	1.29
Isoleucine	1.71	1.85	1.90	1.78
Leucine	2.60	2.76	2.81	2.69
Lysine	2.78	2.91	2.89	2.89
Methionine	0.69	0.72	0.73	0.73
Phenylalanine	1.71	1.81	1.83	1.78
Threonine	1.52	1.61	1.63	1.58
Tryptophan	0.58	0.60	0.60	0.58
Valine	2.60	2.91	3.01	2.81
Selected dispensable AA				
Alanine	2.45	2.76	2.74	2.63
Aspartic acid	3.54	3.69	3.74	3.65
Cysteine	0.33	0.36	0.34	0.35
Glutamic acid	3.79	3.95	3.89	3.88
Glycine	1.97	2.14	2.19	2.06
Proline	2.27	2.41	2.47	2.36
Serine	1.43	1.55	1.56	1.48
Tyrosine	2.58	2.69	2.57	2.73
Taurine	0.08	0.07	0.06	0.07

[1] BSFLA: 0.2% calcium in basal diet + 1.2% $CaCl_2$.
[2] BSFLB: 0.2% calcium in basal + 1.2% $CaCO_3$.
[3] BSFLC: 0.2% calcium in basal diet + 0.75% $CaCO_3$.
[4] BSFLD: 0.2% calcium in basal diet + 0.6% $CaCO_3$ + 0.6% $CaCl_2$.

(4 to 14 wk of age), growing kittens, and adult dogs and cats at maintenance are provided in Table 9. Sulfur AA (methionine + cysteine) was the first-limiting AA for all BSFL for growing puppies and kittens and adult dogs and cats at maintenance, whereas aromatic AA (phenylalanine + tyrosine) was the first-limiting AA for BSFLC and BSFLD for adult cats at maintenance. For these categories, most of the DIAAS-like values for limiting AA were less than 100, suggesting insufficiency if a diet was formulated with only that protein source and at an inclusion level to meet the nutrient profile.

Discussion

BSFL have been of substantial interest over the past couple of decades as a means for organic waste management as well as their ability to convert these food sources into a high-quality nutrient source for livestock feeds and pet foods (Newton et al., 1977, 2005; Sheppard et al., 2002; Tomberlin et al., 2009; Holmes et al., 2012; Kroeckel et al., 2012; Cullere et al., 2016; Marono et al., 2017; Barragan-Fonseca et al., 2018; Chia et al., 2018; Mwaniki et al., 2018; Do et al., 2020; Freel et al., 2021). Similar to mammals, BSFL must be fed a sufficient amount of essential nutrients to meet their nutrient and metabolic requirements (Cohen, 2003). Carbohydrates can be used as an energy source and building block for BSFL tissues. Essential AA such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are important for the production of BSFL tissues, hormones, transport proteins, and energy (Cohen, 2003). Lipids also play several roles in these organisms, such as serving as a rich energy source, as a structural component of cell membranes, and as chemical messengers (Cohen, 2003). Micronutrients are indispensable for BSFL development, but limited information is available in regard to how dietary micronutrients affect BSFL development (Cohen, 2003).

BSFL can be a good source of calcium, but their calcium concentration is very responsive to diet and contain as little as 0.12% Ca (DM basis) up to 6.61% (Dierenfeld and King, 2008; Tschirner and Simon, 2015; Spranghers et al., 2016). If the diet was formulated with 20% of BSFL, the minimal requirement of calcium for kittens and puppies (NRC, 2006) would be met easily (providing 250% and 230% of the requirement). BSFL also have a calcium to phosphorus ratio (2.6:1) that is relatively easy to use in dietary formulations (Finke, 2013). Conversely, tebo worms (11%), Turkestan cockroaches (33%), and house flies (66%) contain low calcium concentrations and calcium and phosphorus ratios (1:18, 1:4.6, and 1:4.9) that are not easy to use in dietary formulas (Finke, 2013). Compared with other insects, BSFL can accumulate high calcium concentrations because they have a mineralized exoskeleton. Therefore, like face flies (*Musca autumnalis*), dietary calcium is positively linked with calcium accumulation in BSFL due to the formation of the cuticle layer during pupation (Roseland et al., 1985; Tomberlin et al., 2002;

Table 3. AA digestibilities (%) of BSFL fed different forms and concentrations of calcium using the precision-fed cecectomized rooster assay

Item	BSFLA[1]	BSFLB[2]	BSFLC[3]	BSFLD[4]	SEM	P-value
Indispensable AA						
Arginine	95.05	93.54	94.58	95.77	0.600	0.6539
Histidine	91.85	90.79	91.43	91.32	0.409	0.8653
Isoleucine	92.53	91.48	92.25	92.75	0.531	0.8740
Leucine	93.06	92.42	92.52	93.47	0.576	0.9271
Lysine	92.28	91.15	91.15	92.25	0.570	0.8438
Methionine	93.71	92.50	93.25	93.52	0.500	0.8515
Phenylalanine	92.80	91.91	92.00	92.47	0.523	0.9397
Threonine	91.62	90.56	90.76	92.11	0.738	0.8910
Tryptophan	95.50	94.40	94.44	95.48	0.411	0.6832
Valine	81.29	83.22	82.00	84.03	1.165	0.8704
Selected dispensable AA						
Alanine	92.69	91.73	92.62	93.29	0.530	0.8137
Aspartic acid	93.68	92.49	93.37	93.57	0.459	0.8278
Cysteine	80.38	76.88	78.08	81.95	1.914	0.8220
Glutamic acid	91.60	89.51	91.44	91.57	0.615	0.6088
Glycine	81.08	79.10	84.72	86.38	1.466	0.2947
Proline	90.31	90.62	91.07	91.67	0.543	0.8576
Serine	89.52	87.92	89.37	91.18	0.939	0.7240
Tyrosine	93.66	93.26	92.64	93.85	0.395	0.7535

[1] BSFLA: 0.2% calcium in basal diet + 1.2% $CaCl_2$.
[2] BSFLB: 0.2% calcium in basal + 1.2% $CaCO_3$.
[3] BSFLC: 0.2% calcium in basal diet + 0.75% $CaCO_3$.
[4] BSFLD: 0.2% calcium in basal diet + 0.6% $CaCO_3$ + 0.6% $CaCl_2$.

Table 4. DIAAS-like reference values[1] of BSFL fed different forms and concentrations of calcium for growing and reproducing dogs and growing puppies[2]

	AAFCO (2019)[3]					NRC (2006)[3]				
Item	BSFLA	BSFLB	BSFLC	BSFLD	SEM	BSFLA	BSFLB	BSFLC	BSFLD	SEM
Indispensable AA										
Arginine	98.51^{b}	104.32^{ab}	103.39^{ab}	105.03^{a}	0.906	124.70^{b}	132.05^{ab}	130.88^{ab}	132.95^{a}	1.147
Histidine	139.73^{b}	146.86^{a}	147.31^{a}	146.79^{a}	1.030	157.65^{b}	165.69^{a}	166.20^{a}	165.61^{a}	1.162
Isoleucine	120.30^{a}	133.59^{a}	133.60^{a}	127.50^{a}	1.600	131.41^{b}	145.92^{a}	145.93^{a}	139.27^{a}	1.748
Leucine	101.25^{b}	110.81^{a}	109.07^{a}	106.87^{ab}	1.148	101.25^{b}	110.81^{a}	109.07^{a}	106.87^{ab}	1.148
Lysine	153.87^{b}	165.17^{a}	158.41^{ab}	162.42^{ab}	1.460	157.37^{b}	168.93^{a}	162.01^{ab}	166.11^{ab}	1.493
Methionine	99.72^{b}	106.59^{a}	105.25^{a}	106.94^{a}	0.930	105.77^{b}	113.05^{a}	111.63^{a}	113.43^{a}	0.986
Met + Cys	49.89	51.38	51.51	53.24	0.751	49.89	51.38	51.51	53.24	0.752
Phenylalanine	103.20^{b}	112.33^{a}	109.77^{a}	108.74^{ab}	1.063	131.78^{b}	143.44^{a}	140.17^{a}	138.85^{ab}	1.357
Phe + Tyr	121.01^{b}	126.07^{ab}	122.91^{ab}	127.13^{a}	0.867	121.01^{b}	126.07^{ab}	122.91^{ab}	127.13^{a}	0.867
Threonine	72.28^{b}	78.57^{a}	76.98^{ab}	76.73^{ab}	0.862	92.80^{b}	100.88^{a}	98.84^{ab}	98.52^{ab}	1.106
Tryptophan	149.50^{b}	158.72^{a}	153.32^{ab}	151.82^{b}	1.085	130.00^{b}	138.02^{a}	133.32^{ab}	132.01^{b}	0.944
Valine	167.77^{b}	199.59^{a}	196.42^{a}	190.39^{ab}	4.174	167.77^{b}	199.59^{a}	196.42^{a}	190.39^{ab}	4.174

[1] DIAAS-like reference values were calculated from the true digestibility of AA in cecectomized roosters.
[2] DIAAS-like reference values were calculated using the AAFCO (2019) nutrient profiles of AA for growth and reproduction of dogs and NRC (2006) recommended allowances of AA for growing puppies (4 to 14 wk of age).
[3] BSFLA: 0.2% calcium in basal diet + 1.2% $CaCl_2$; BSFLB: 0.2% calcium in basal + 1.2% $CaCO_3$; BSFLC: 0.2% calcium in basal diet + 0.75% $CaCO_3$; BSFLD: 0.2% calcium in basal diet + 0.6% $CaCO_3$ + 0.6% $CaCl_2$.
[a,b] Within a row, means lacking a common superscript letter differ ($P < 0.05$); *n*, 4 roosters per treatment.

Finke, 2013). For those reasons, dietary calcium source may be an important consideration for BSFL development.

The gastrointestinal tract of BSFL is composed of three parts, including the foregut, midgut, and hindgut, with the midgut serving as the most important section for nutrient absorption (Kim et al., 2011). The Malpighian tubules located in the midgut and hindgut junction are essential for the excretion of nitrogenous products and other metabolites and maintaining nutrient balance (Murakami and Shiotsuki, 2001; Chapman, 2013; Gold et al., 2018). According to Grodowitz et al. (1987) and Krueger et al. (1988), dietary calcium in face flies is transported from the Malpighian tubules to the cuticle via hemolymph. They have mineralization of the epidermis during the pupal stage because mineralized granules, which are composed of calcium, magnesium, phosphate, and carbonate, accumulate in the lumen of the larval Malpighian tubules. Therefore, as with face flies, BSFL may have a similar mechanism of dietary calcium absorption.

Table 5. DIAAS-like reference values[1] of BSFL fed different forms and concentrations of calcium for growing and reproducing cats and growing kittens[2]

	AAFCO (2019)[3]					NRC (2006)[3]				
Item	BSFLA	BSFLB	BSFLC	BSFLD	SEM	BSFLA	BSFLB	BSFLC	BSFLD	SEM
Indispensable AA										
Arginine	105.93^{b}	112.17^{ab}	111.18^{ab}	112.93^{a}	0.974	114.02^{b}	120.74^{ab}	119.67^{ab}	121.56^{a}	1.049
Histidine	248.41^{b}	261.09^{a}	261.89^{a}	260.96^{a}	1.831	207.01^{b}	217.58^{a}	218.24^{a}	217.47^{a}	1.526
Isoleucine	203.37^{b}	225.83^{a}	225.85^{a}	215.53^{a}	2.705	175.75^{b}	195.17^{a}	195.18^{a}	186.26^{a}	2.337
Leucine	136.05^{b}	148.91^{a}	146.57^{a}	143.61^{ab}	1.542	113.38^{b}	124.09^{a}	122.14^{a}	119.67^{ab}	1.285
Lysine	153.87^{b}	165.17^{a}	158.41^{ab}	162.42^{ab}	1.460	181.03^{b}	194.32^{a}	186.36^{ab}	191.09^{ab}	1.718
Methionine	75.06^{b}	80.23^{a}	79.22^{a}	80.50^{a}	0.700	88.14^{b}	94.21^{b}	93.03^{a}	94.52^{a}	0.822
Met + Cys	42.33	43.60	43.70	45.18	0.638	44.10	45.41	45.52	47.06	0.664
Phenylalanine	219.63^{b}	239.06^{a}	233.62^{a}	231.42^{ab}	2.261	190.35^{b}	207.19^{a}	202.47^{a}	200.56^{ab}	1.960
Phe + Tyr	109.25^{b}	113.81^{ab}	110.96^{ab}	114.77^{a}	0.782	91.52^{b}	95.34^{ab}	92.95^{ab}	96.15^{a}	0.655
Threonine	137.30^{b}	149.25^{a}	146.23^{ab}	145.75^{ab}	1.637	128.50^{b}	139.68^{a}	136.86^{ab}	136.41^{ab}	1.532
Tryptophan	159.46^{b}	169.30^{a}	163.54^{ab}	161.94^{b}	1.158	207.63^{b}	220.44^{a}	212.95^{ab}	210.86^{b}	1.507
Valine	237.67^{b}	282.75^{a}	278.27^{a}	269.72^{ab}	5.913	198.06^{b}	235.63^{a}	231.89^{a}	224.76^{ab}	4.928

[1] DIAAS-like reference values were calculated from the true digestibility of AA in cecectomized roosters.

[2] DIAAS-like reference values were calculated using the AAFCO (2019) nutrient profiles of AA for growth and reproduction of cats and NRC (2006) recommended allowances of AA for growing kittens.

[3] BSFLA: 0.2% calcium in basal diet + 1.2% $CaCl_2$; BSFLB: 0.2% calcium in basal + 1.2% $CaCO_3$; BSFLC: 0.2% calcium in basal diet + 0.75% $CaCO_3$; BSFLD: 0.2% calcium in basal diet + 0.6% $CaCO_3$ + 0.6% $CaCl_2$.

[a,b] Within a row, means lacking a common superscript letter differ ($P < 0.05$); *n*, 4 roosters per treatment.

Table 6. DIAAS-like reference values[1] of BSFL fed different forms and concentrations of calcium for adult dogs[2]

	AAFCO (2019)[3]					NRC (2006)[3]				
Item	BSFLA	BSFLB	BSFLC	BSFLD	SEM	BSFLA	BSFLB	BSFLC	BSFLD	SEM
Indispensable AA										
Arginine	154.53^{b}	163.64^{ab}	162.19^{ab}	164.75^{a}	1.422	125.09^{b}	132.48^{ab}	131.29^{ab}	133.37^{a}	1.151
Histidine	258.87^{b}	272.08^{a}	272.92^{a}	271.95^{a}	1.901	143.81^{b}	151.16^{a}	151.62^{a}	151.08^{a}	1.060
Isoleucine	179.82^{b}	199.69^{a}	199.70^{a}	190.57^{a}	2.392	99.90^{b}	110.94^{a}	110.94^{a}	105.87^{a}	1.329
Leucine	153.66^{b}	168.18^{a}	165.53^{a}	162.19^{ab}	1.742	85.37^{b}	93.43^{a}	91.96^{a}	90.11^{ab}	0.968
Lysine	175.85^{b}	188.77^{a}	181.04^{ab}	185.63^{ab}	1.669	175.85^{b}	188.77^{a}	181.04^{ab}	185.63^{ab}	1.669
Methionine	84.61^{b}	90.44^{a}	89.31^{a}	90.74^{a}	0.789	47.01^{b}	50.24^{a}	49.61^{a}	50.41^{a}	0.438
Met + Cys	42.98	44.27	44.37	45.87	0.648	23.88	24.59	24.65	25.48	0.360
Phenylalanine	152.28^{b}	165.75^{a}	161.98^{a}	160.45^{ab}	1.568	84.60^{b}	92.08^{a}	89.99^{a}	89.14^{ab}	0.871
Phe + Tyr	170.07^{b}	177.18^{ab}	172.74^{ab}	178.68^{a}	1.218	94.49^{b}	98.43^{ab}	95.96^{ab}	99.26^{a}	0.677
Threonine	125.28^{b}	136.19^{a}	133.44^{ab}	133.00^{ab}	1.494	77.70^{b}	84.46^{a}	82.75^{ab}	82.48^{ab}	0.926
Tryptophan	149.50^{b}	158.72^{a}	153.32^{ab}	151.82^{b}	1.085	94.92^{b}	100.77^{a}	97.35^{ab}	96.39^{b}	0.689
Valine	186.26^{b}	221.59^{a}	218.07^{a}	211.37^{ab}	4.634	103.48^{b}	123.10^{a}	121.15^{a}	117.43^{ab}	2.574

[1] DIAAS-like reference values were calculated from the true digestibility of AA in cecectomized roosters.

[2] DIAAS-like reference values were calculated using the AAFCO (2019) nutrient profiles and NRC (2006) recommended allowances of AA for adult dogs at maintenance.

[3] BSFLA: 0.2% calcium in basal diet + 1.2% $CaCl_2$; BSFLB: 0.2% calcium in basal + 1.2% $CaCO_3$; BSFLC: 0.2% calcium in basal diet + 0.75% $CaCO_3$; BSFLD: 0.2% calcium in basal diet + 0.6% $CaCO_3$ + 0.6% $CaCl_2$.

[a,b] Within a row, means lacking a common superscript letter differ ($P < 0.05$); *n*, 4 roosters per treatment.

There are many potential calcium sources in animal feeds, such as organic salts (tricalcium citrate, calcium lactate, calcium lactate gluconate, and calcium gluconate) and inorganic salts (calcium chloride, calcium carbonate, and calcium phosphate; Trailokya et al., 2017). In the current study, we compared $CaCO_3$ and $CaCl_2$ as dietary calcium sources for BSFL and they have different solubilities (insoluble vs. soluble; pH range between 3 and 6), calcium concentrations (40% vs. 27%), and acid-binding capacities (244 vs. 2.4 mEq/kg to reach pH of 3) (Weaver, 1998; Hamdi et al., 2015). Several studies have determined that calcium citrate (soluble) has a higher absorption rate than $CaCO_3$ (insoluble) due to its solubility in water (Nicar and Pak, 1985; Heller et al., 1999). Heaney et al. (1990), however, tested seven different calcium salts (calcium oxalate, hydroxyapatite, tricalcium phosphate, calcium citrate, calcium citrate malate, bisglycinocalcium, and $CaCO_3$) to evaluate their relationship between solubility and absorption and were unable to identify a significant correlation. Another in vitro study conducted by Goss et al. (2007) reported that while $CaCO_3$ (3.6 mg mL^{-1}) had a greater solubility in the gastrointestinal tract than calcium citrate (0.2 mg mL^{-1}) at a pH of 6, $CaCO_3$ (0.12 mg mL^{-1}) had a lower solubility than calcium citrate (0.24 mg mL^{-1}) at a pH of 7.5. The solubility of dietary calcium in water is affected by pH because it impacts its form (anion vs. cation). Secretion of hydrochloric acid and sodium bicarbonate from the stomach and intestines affects the pH in the gastrointestinal tract. Thus,

Table 7. DIAAS-like reference values[1] of BSFL fed different forms and concentrations of calcium for adult cats[2]

	AAFCO (2019)[3]					NRC (2006)[3]				
Item	BSFLA	BSFLB	BSFLC	BSFLD	SEM	BSFLA	BSFLB	BSFLC	BSFLD	SEM
Indispensable AA										
Arginine	109.46^{b}	115.91^{ab}	114.88^{ab}	116.70^{a}	1.001	113.72^{b}	120.43^{ab}	119.36^{ab}	121.24^{a}	1.046
Histidine	229.17^{b}	240.88^{a}	241.61^{a}	240.76^{a}	1.689	210.19^{b}	220.92^{a}	221.60^{a}	220.82^{a}	1.549
Isoleucine	189.81^{b}	210.78^{a}	210.79^{a}	201.16^{a}	2.524	176.57^{b}	196.07^{a}	196.09^{a}	187.13^{a}	2.348
Leucine	121.72^{b}	133.22^{a}	131.12^{a}	128.47^{ab}	1.380	113.82^{b}	124.58^{a}	122.62^{a}	120.14^{ab}	1.290
Lysine	192.80^{b}	206.96^{a}	198.48^{ab}	203.52^{ab}	1.829	362.05^{b}	388.64^{a}	372.72^{ab}	382.17^{ab}	3.435
Methionine	201.67^{b}	215.54^{a}	212.84^{a}	216.27^{a}	1.880	182.50^{b}	195.06^{a}	192.62^{a}	195.71^{a}	1.701
Met + Cys	100.89	103.91	104.16	107.67	1.520	91.31	94.03	94.26	97.44	1.375
Phenylalanine	247.45^{b}	269.34^{a}	263.21^{a}	260.73^{ab}	2.548	190.35^{b}	207.19^{a}	202.47^{a}	200.56^{ab}	1.960
Phe + Tyr	118.82^{b}	123.78^{ab}	120.68^{ab}	124.83^{a}	0.851	91.40^{b}	95.22^{ab}	92.83^{ab}	96.02^{a}	0.655
Threonine	118.99^{b}	129.35^{a}	126.73^{ab}	126.32^{ab}	1.419	128.50^{b}	139.68^{a}	136.86^{ab}	136.41^{ab}	1.532
Tryptophan	215.94^{b}	229.26^{a}	221.46^{ab}	219.29^{b}	1.568	204.44^{b}	217.05^{a}	209.67^{ab}	207.61^{b}	1.484
Valine	212.63^{b}	252.96^{a}	248.94^{a}	241.29^{ab}	5.290	198.84^{b}	236.55^{a}	232.80^{a}	225.65^{ab}	4.947

[1] DIAAS-like reference values were calculated from the true digestibility of AA in cecectomized roosters.
[2] DIAAS-like reference values were calculated using the AAFCO (2019) nutrient profiles and NRC (2006) recommended allowances of AA for adult cats at maintenance.
[3] BSFLA: 0.2% calcium in basal diet + 1.2% $CaCl_2$; BSFLB: 0.2% calcium in basal + 1.2% $CaCO_3$; BSFLC: 0.2% calcium in basal diet + 0.75% $CaCO_3$; BSFLD: 0.2% calcium in basal diet + 0.6% $CaCO_3$ + 0.6% $CaCl_2$.
[a,b] Within a row, means lacking a common superscript letter differ ($P < 0.05$); *n*, 4 roosters per treatment.

Table 8. First-limiting AA based on DIAAS-like reference values[1] of BSFL fed different forms and concentrations of calcium from AAFCO (2019) nutrient profiles[2]

	Growth and reproduction		Adults	
Item[3]	Dogs	Cats	Dogs	Cats
BSFLA	50 (Met + Cys)	42 (Met + Cys)	43 (Met + Cys)	101 (Met + Cys)
BSFLB	51 (Met + Cys)	44 (Met + Cys)	44 (Met + Cys)	104 (Met + Cys)
BSFLC	51 (Met + Cys)	44 (Met + Cys)	44 (Met + Cys)	104 (Met + Cys)
BSFLD	53 (Met + Cys)	45 (Met + Cys)	45 (Met + Cys)	108 (Met + Cys)

[1] DIAAS-like values were calculated from the true digestibility of AA in cecectomized roosters.
[2] DIAAS-like values were calculated using the AAFCO (2019) nutrient profiles of AA for dogs and cats.
[3] BSFLA: 0.2% calcium in basal diet + 1.2% $CaCl_2$; BSFLB: 0.2% calcium in basal + 1.2% $CaCO_3$; BSFLC: 0.2% calcium in basal diet + 0.75% $CaCO_3$; BSFLD: 0.2% calcium in basal diet + 0.6% $CaCO_3$ + 0.6% $CaCl_2$.

Table 9. First-limiting AA based on DIAAS-like reference values[1] of BSFL fed different forms and concentrations of calcium from NRC (2006) recommended allowances[2]

			Adult	
Item[3]	Puppies (4 to 14 wk of age)	Kittens	Dogs	Cats
BSFLA	50 (Met + Cys)	44 (Met + Cys)	24 (Met + Cys)	91 (Met + Cys)
BSFLB	51 (Met + Cys)	45 (Met + Cys)	25 (Met + Cys)	94 (Met + Cys)
BSFLC	52 (Met + Cys)	46 (Met + Cys)	25 (Met + Cys)	93 (Phe + Tyr)
BSFLD	53 (Met + Cys)	47 (Met + Cys)	25 (Met + Cys)	96 (Phe + Tyr)

[1] DIAAS-like values were calculated from the true digestibility of AA in cecectomized roosters.
[2] DIAAS-like values were calculated using the NRC (2006) recommended allowances of AA for dogs and cats.
[3] BSFLA: 0.2% calcium in basal diet + 1.2% $CaCl_2$; BSFLB: 0.2% calcium in basal + 1.2% $CaCO_3$; BSFLC: 0.2% calcium in basal diet + 0.75% $CaCO_3$; BSFLD: 0.2% calcium in basal diet + 0.6% $CaCO_3$ + 0.6% $CaCl_2$.

the solubility values determined from the gastrointestinal tract would be a key factor to understand the effect of the solubility on calcium absorption rather than simple aqueous solubility. Dietary calcium sources used in the current study have different pH-dependent solubilities and may have different absorption rates in the midgut of BSFL. The pH of the BSFL midgut changes according to region (anterior midgut: pH = 7, mid-midgut: pH = 2, and posterior midgut: pH = 6.3 to 9.3; Espinoza-Fuentes and Terra, 1987; Overend et al., 2016; Gold et al., 2018). Although $CaCO_3$ has a higher calcium content than $CaCl_2$, $CaCl_2$ may be more bioavailable to BSFL because of its higher solubility in the midgut, and, in this trial, the combination of both forms resulted in the highest numerical concentration of BSFL calcium.

The nutrient concentrations, including indispensable AA, were similar for all BSFL tested in the current study. Shumo et al. (2019) reared BSFL on three different organic wastes, including chicken manure (high ash: 20% and low fat: 2.7%), brewer's spent grain (high neutral detergent fiber [**NDF**]: 50% and high acid detergent fiber [**ADF**]: 39%), and kitchen waste (high protein: 20%). BSFL fed kitchen waste had higher DM (87.5%) and fat (34.3%) concentrations and lower CP (33%) concentrations than BSFL fed chicken manure or brewer's spent grain. Also, BSFL reared on brewer's spent grain had higher ADF (15%) and NDF (28.6%) concentrations than larvae fed chicken manure (ADF: 12.6% and NDF: 21.9%) or kitchen waste (ADF: 13.2% and NDF: 20.4%). Barragan-Fonseca et al. (2019) reported that BSFL CP concentration increased when larvae were fed a diet containing 10% CP concentration compared with a high-protein diet (17%). Similarly, Tschirner and Simon (2015) reported that larvae fed a high-fiber diet containing only 8.5% CP had a higher CP content (52.3%) than larvae (44.6%) fed a high-protein diet (31.2% CP).

BSFL crude fat concentration was shown to increase as dietary carbohydrate concentration increased (Barragan-Fonseca et al., 2019). Compared with AA concentrations of BSFL reported by Shumo et al. (2019), BSFL fed kitchen waste had higher concentrations of indispensable AA (arginine and phenylalanine) and dispensable AA (proline, hydro-proline, and tyrosine) than BSFL fed chicken manure or brewer's spent grain. Based on the previous studies, the nutrient composition (DM, CP, fat, ADF, and NDF) of the substrates affected the chemical composition and AA concentration of BSFL. In the current study, different forms and concentrations of dietary calcium sources influenced the calcium concentrations of BSFL, but there was no significant difference in the nutrient composition of BSFL among treatments.

The precision-fed cecectomized rooster assay is often used to test the protein quality of pet food ingredients because the AA digestibilities and response patterns have been shown to be similar to that of ileal-cannulated dogs (r = 0.87 to 0.92; Johnson et al., 1998; Faber et al., 2010; Kerr et al., 2014; Oba et al., 2019). Other methods of testing AA digestibilities have drawbacks when compared with the cecectomized rooster assay. For example, the total collection method that uses fecal analysis is heavily influenced by the fermentative activity of the large intestinal microbiota and is a time-consuming, labor-intensive, and expensive method (Hendriks and Sritharan, 2002; Hendriks et al., 2013). Intestinal cannulas have been used to estimate nutrient digestion at specific points in the gastrointestinal tract for many years. Cannulation, however, also has many disadvantages, such as leakage of chyme from the cannula, skin ulceration and infection, and the discomfort of animals (Hill et al., 1996). Therefore, the cecectomized rooster assay is an appropriate model to evaluate the protein quality of ingredients because it minimizes the influence of microbes in the hindgut.

Because BSFL have a high calcium concentration due to their mineralized exoskeleton (Grodowitz and Broce, 1983; Krueger et al., 1988) and high dietary calcium concentrations may influence the digestibility of nitrogen and AA (Selle et al., 2009; Wilkinson et al., 2014), the current study was conducted to test whether dietary calcium source or concentration affected nutrient digestibility. It was interesting to note that nutrient and AA digestibilities were similar among all BSFL tested in the current study. According to McDonald and Solvyns (1964), increasing dietary calcium ($CaCO_3$) concentrations from 9 to 25 g/kg resulted in a greater small intestinal pH from 5.6 to 6.0 and a reduction in weight gain of chickens from 77 to 63.5 g/d. Wilkinson et al. (2014) demonstrated that increasing dietary calcium concentration ($CaCO_3$) from 2.5 to 10 g/kg led to a reduction in the apparent ileal digestibility of DM, nitrogen, and AA in broiler chickens. The possible reason for these results is that the increased intestinal pH resulting from the high dietary calcium concentrations reduced the action of pepsin. In the current study, the calcium form and concentration fed to BSFL had no adverse effects on BSFL protein quality or calcium concentrations, which may have been due to a low acid-binding capacity, impact on intestinal pH, and consequent nutrient absorption.

DIAAS-like reference values are an indicator of protein quality, providing a more accurate measure than the protein digestibility-corrected AA score because it uses ileal rather than fecal digestibility in its calculation (FAO, 2011; Mathai et al., 2017; Oba et al., 2019). Based on the DIAAS-like reference values in the current study, all BSFL tested seem to provide sufficient AA for growing puppies and kittens except for threonine and sulfur AA (methionine + cysteine). Because the AAFCO and NRC recommended allowances are lower for adult dogs and cats, more AA would be insufficient for those life stages if diets were only formulated using BSFL as the sole protein source and at a rate to meet the recommended CP concentrations.

This study had a couple of limitations that should be discussed briefly. First, although the precision-fed cecectomized rooster assay is a highly repeatable method that has been used to study many novel proteins in recent years (Kerr et al., 2013, 2014; Deng et al., 2016; Oba et al., 2019; Do et al., 2020), the number of replications in this study (4 to 5 roosters per treatment) was low. This replication number may have provided us with lower than ideal statistical power and limited our ability to detect differences among treatments. Also, the DIAAS-like values used to estimate protein quality for dog and cat foods are based on the assumption that diets would be formulated with that single protein source and contain an inclusion level to meet the CP recommendation. Dog or cat foods formulated using a different strategy would need to keep this in mind when assessing the value of BSFL.

In conclusion, our data show that BSFL contain a relatively high concentration of calcium and that BSFLD fed $CaCl_2$ and $CaCO_3$ accumulated more calcium than the other BSFL. Despite their differences in nutrient composition, nutrient and AA digestibilities among all BSFL sources were similar when tested in the precision-fed cecectomized rooster assay. Sulfur AA (methionine + cysteine) and aromatic AA (phenylalanine + tyrosine) were estimated to be the first-limiting AA of BSFL based on DIAAS-like reference values for dogs and cats. All BSFL ingredients, however, showed very high AA digestibilities, with most exceeding 90%. Therefore, although dietary calcium form and concentration may affect the calcium concentrations of BSFL, they all serve as high-quality protein sources for use in livestock feeds and pet foods.

Acknowledgment

The funding for this study was provided by EnviroFlight.

Conflict of interest statement

L.K. is employed by EnviroFlight. All other authors have no conflicts of interest.

Literature Cited

American Association of Cereals Chemists (AACC). 1983. *Approved methods*. 8th ed. Saint Paul (MN): American Association of Cereal Chemists.

Association of American Feed Control Officials (AAFCO). 2019. *Official publication*. Oxford (IN): AAFCO.

Association of Official Analytical Chemists (AOAC). 2006. *Official methods of analysis*. 17th ed. Gaithersburg (MD): Association of Official Analytical Chemists.

Barragan-Fonseca, K. B., M. Dicke, and J. J. A. van Loon. 2018. Influence of larval density and dietary nutrient concentration on performance, body protein, and fat contents of black soldier fly larvae (*Hermetia illucens*). *Entomol. Exp. Appl.* **166**:761–770. doi:10.1111/eea.12716

Barragan-Fonseca, K. B., G. Gort, M. Dicke, and J. J. A. van Loon. 2019. Effects of dietary protein and carbohydrate on life-history traits and body protein and fat contents of the black soldier fly (*Hermetia illucens*). *Physiol. Entomol.* **44**:148–159. doi:10.1111/phen.12285

Bosch, G., S. Zhang, D. G. Oonincx, and W. H. Hendriks. 2014. Protein quality of insects as potential ingredients for dog and cat foods. *J. Nutr. Sci.* **3**:e29. doi:10.1017/jns.2014.23

Budde, E. F. 1952. The determination of fat in baked biscuit type of dog foods. *J. Assoc. Off. Agric. Chem.* **35**:799–805. doi:10.1093/jaoac/35.3.799

Chapman, R. F. 2013. *The insects: structure and function*. Cambridge (UK): Cambridge University Press.

Chia, S. Y., C. M. Tanga, F. M. Khamis, S. A. Mohamed, D. Salifu, S. Sevgan, K. K. M. Fiaboe, S. Niassy, J. J. A. van Loon, M. Dicke, *et al.* 2018. Threshold temperatures and thermal requirements of black soldier fly *Hermetia illucens*: implications for mass production. *PLoS One* **13**:e0206097. doi:10.1371/journal.pone.0206097

Cohen, A. C. 2003. *Insect Diets. Science and Technology*. 2nd ed. London (UK): CRC Press.

Cullere, M., G. Tasoniero, V. Giaccone, R. Miotti-Scapin, E. Claeys, S. De Smet, and A. Dalle Zotte. 2016. Black soldier fly as dietary protein source for broiler quails: apparent digestibility, excreta microbial load, feed choice, performance, carcass and meat traits. *Animal* **24**:1–8. doi:10.1017/S1751731116001270

Deng, P., P. L. Utterback, C. M. Parsons, L. Hancock, and K. S. Swanson. 2016. Chemical composition, true nutrient digestibility, and true metabolizable energy of novel pet food protein sources using the precision-fed cecectomized rooster assay. *J. Anim. Sci.* **94**:3335–3342. doi:10.2527/jas.2016-0473

Diener, S., C. Zurbrugg, and K. Tockner. 2009. Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. *Waste Manage. Res.* **27**:603–610. doi:10.1177/0734242X09103838

Dierenfeld, E. S., and J. King. 2008. Digestibility and mineral availability of phoenix worms, *Hermetia illucens*, ingested by mountain chicken frogs, *Leptodactylus fallax*. *J. Herpetol. Med. Surg.* **18**:100–105. doi:10.5818/1529-9651.18.3-4.100

Do, S., L. Koutsos, P. L. Utterback, C. M. Parsons, M. R. C. de Godoy, and K. S. Swanson. 2020. Nutrient and AA digestibility of black soldier fly larvae differing in age using the precision-fed cecectomized rooster assay. *J. Anim. Sci.* **98**:1–10. doi:10.1093/jas/skz363

Espinoza-Fuentes, F. P., and W. R. Terra. 1987. Physiological adaptations for digesting bacteria – water fluxes and distribution of digestive enzymes in *Musca domestica* larval midgut. *Insect Biochem.* **17**:809–817. doi:10.1016/0020-1790(87)90015-1

Faber, T. A., P. J. Bechtel, D. C. Hernot, C. M. Parsons, K. S. Swanson, S. Smiley, and G. C. Fahey Jr. 2010. Protein digestibility evaluations of meat and fish substrates using laboratory, avian, and ileally cannulated dog assays. *J. Anim. Sci.* **88**:1421–1432. doi:10.2527/jas.2009-2140

Finke, M. D. 2013. Complete nutrient content of four species of feeder insects fed enhanced diets during growth. *Zoo Biol.* **34**:554–64. doi:10.1002/zoo.21246

Food and Agriculture Organization (FAO). 2011. *Dietary protein quality evaluation in human nutrition*. Auckland (New Zealand): Food and Agriculture Organization of the United Nations.

Freel, T. A., A. McComb, and E. A. Koutsos. 2021. Digestibility and safety of dry black soldier fly larvae meal and black soldier fly larvae oil in dogs. *J. Anim. Sci.* **99**:1–8. doi:10.1093/jas/skab047

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

Goss, S. L., K. A. Lemons, J. E. Kerstetter, and R. H. Bogner. 2007. Determination of calcium salt solubility with changes in pH and $P_{(CO(2))}$, simulating varying gastrointestinal environments. *J. Pharm. Pharmacol.* **59**:1485–1492. doi:10.1211/jpp.59.11.0004

Grodowitz, M. J., and A. B. Broce. 1983. Calcium storage in face fly (Diptera: Muscidae) larvae for puparium formation. *Ann. Entomol. Soc. Am.* **75**:418–424. doi:10.1093/aesa/76.3.418

Grodowitz, M. J., A. B. Broce, and K. J. Kramer. 1987. Morphological and biochemical composition of mineralized granules from the Malpighian tubules of *Musca autumnalis* de geer larvae (Diptera: Muscidae). *Insect Biochem.* **17**:335–345. doi:10.1016/0020-1790(87)90077-1

Hamdi, M., D. Solà-Oriol, R. Davin, and J. F. Perez. 2015. Calcium sources and their interaction with the different levels of non-phytate phosphorus affect performance and bone mineralization in broiler chickens. *Poult. Sci.* **94**:2136–2143. doi:10.3382/ps/peu061

Heaney, R. P., R. R. Recker, and C. M. Weaver. 1990. Absorbability of calcium sources: the limited role of solubility. *Calcif. Tissue Int.* **46**:300–304. doi:10.1007/BF02563819

Heller, H. J., A. Stewart, S. Haynes, and C. Y. Pak. 1999. Pharmacokinetics of calcium absorption from two commercial calcium supplements. *J. Clin. Pharmacol.* **39**:1151–1154. doi:10.1177/009127009903901106

Hendriks, W. H., and K. Sritharan. 2002. Apparent ileal and fecal digestibility of dietary protein is different in dogs. *J. Nutr.* **132**(6 Suppl 2):1692S–1694S. doi:10.1093/jn/132.6.1692S

Hendriks, W. H., D. G. Thomas, G. Bosch, and G. C. Fahey, Jr. 2013. Comparison of ileal and total tract nutrient digestibility of dry dog foods. *J. Anim. Sci.* **91**:3807–3814. doi:10.2527/jas.2012-5864

Hill, R. C., G. W. Ellison, C. F. Burrows, J. E. Bauer, and B. Carbia. 1996. Ileal cannulation and associated complications in dogs. *Lab. Anim. Sci.* **46**:77–80.

Holmes, L. A., S. L. Vanlaerhoven, and J. K. Tomberlin. 2012. Relative humidity effects on the life history of *Hermetia illucens* (Diptera: Stratiomyidae). *Environ. Entomol.* **41**:971–978. doi:10.1603/EN12054

Johannsen, O. A. 1922. Stratiomyiid larvae and puparia of the north eastern states. *J. N. Y. Entomol. Soc.* **30**:141–153.

Johnson, M. L., C. M. Parsons, G. C. Fahey Jr, N. R. Merchen, and C. G. Aldrich. 1998. Effects of species raw material source, ash content, and processing temperature on amino acid digestibility of animal by-product meals by cecectomized roosters and ileally cannulated dogs. *J. Anim. Sci.* **76**:1112–1122. doi:10.2527/1998.7641112x

Kerr, K. R., A. N. Beloshapka, C. L. Morris, C. M. Parsons, S. L. Burke, P. L. Utterback, and K. S. Swanson. 2013. Evaluation of four raw meat diets using domestic cats, captive exotic felids, and cecectomized roosters. *J. Anim. Sci.* **91**:225–237. doi:10.2527/jas.2011-4835

Kerr, K. R., K. L. Kappen, L. M. Garner, P. L. Utterback, C. M. Parsons, and K. S. Swanson. 2014. Commercially available avian and mammalian whole prey diet items targeted for consumption by managed exotic and domestic pet felines: true metabolizable energy and amino acid digestibility using the precision-fed cecectomized rooster assay. *J. Anim. Sci.* **92**:4478–4485. doi:10.2527/jas.2013-7246

Kim, W., S. Bae, K. Park, S. Lee, Y. Choi, S. Han, and Y. Koh. 2011. Biochemical characterization of digestive enzymes in the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). *J. Asia Pac. Entomol.* **14**:11–14. doi:10.1016/j.aspen.2010.11.003

Kroeckel, S., A. G. E. Harjes, I. Roth, H. Katz, S. Wuertz, A. Susenbeth, and C. Schulz. 2012. When a turbot catches a fly: evaluation of a pre-pupae meal of the black soldier fly (*Hermetia illucens*) as fish meal substitute — growth performance and chitin degradation in juvenile turbot (*Psetta maxima*). *Aquacult. Fish. Manage.* **364–365**:345–352. doi:10.1016/j.aquaculture.2012.08.041

Krueger, R. A., A. B. Broce, T. L. Hopkins, and K. J. Kramer. 1988. Calcium transport from Malpighian tubules to puparial cuticle of *Musca autumnalis*. *J. Comp. Physiol. B* **158**:413–419. doi:10.1007/BF00691138

Lock, E., T. Arsiwalla, and R. Waagbø. 2015. Insect larvae meal as an alternative source of nutrients in the diet of Atlantic salmon (*Salmo salar*) post smolt. *Aquacult. Nutr.* **22**:1202–1213. doi:10.1111/anu.12343

Marono, S., R. Loponte, P. Lombardi, G. Vassalotti, M. E. Pero, F. Russo, L. Gasco, G. Parisi, G. Piccolo, S. Nizza, *et al.* 2017. Productive performance and blood profiles of laying hens fed *Hermetia illucens* larvae meal as total replacement of soybean meal from 24 to 45 weeks of age. *Poult. Sci.* **96**:1783–1790. doi:10.3382/ps/pew461

Mathai, J. K., Y. Liu, and H. H. Stein. 2017. Values for digestible indispensable amino acid scores (DIAAS) for some dairy and plant proteins may better describe protein quality than values calculated using the concept for protein digestibility-corrected amino acid scores (PDCAAS). *Br. J. Nutr.* **117**:490–499. doi:10.1017/S0007114517000125

McDonald, M. W., and A. Solvyns. 1964. Dietary calcium levels and chicken growth. Proceedings of the Australasian Poultry Science Convention; May 25 to 29, 1964; Queensland, Australia.

Sydney (Australia): World's Poultry Science Association; p. 112–116.

Murakami, R., and Y. Shiotsuki. 2001. Ultrastructure of the hindgut of *Drosophila* larvae, with special reference to the domains identified by specific gene expression patterns. *J. Morphol.* **248**:144–150. doi:10.1002/jmor.1025

Mwaniki, Z., M. Neijat, and E. Kiarie. 2018. Egg production and quality responses of adding up to 7.5% defatted black soldier fly larvae meal in a corn-soybean meal diet fed to Shaver White Leghorns from wk 19 to 27 of age. *Poult. Sci.* **97**:2829–2835. doi:10.3382/ps/pey118

National Research Council (NRC). 2006. *Nutrient requirements of dogs and cats*. Washington (DC): National Academic Press.

Nguyen, T. T., J. K. Tomberlin, and S. Vanlaerhoven. 2015. Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. *Environ. Entomol.* **44**:406–410. doi:10.1093/ee/nvv002

Nicar, M. J., and C. Y. Pak. 1985. Calcium bioavailability from calcium carbonate and calcium citrate. *J. Clin. Endocrinol. Metab.* **61**:391–393. doi:10.1210/jcem-61-2-391

Newton, G. L., C. V. Booram, R. W. Barker, and O. M. Hale. 1977. Dried *Hermetia illucens* larvae meal as a supplement for swine. *J. Anim. Sci.* **44**:395–400. doi:10.2527/jas1977.443395x

Newton, L., C. Sheppard, D. W. Watson, G. Burtle, and R. Dove. 2005. Using the black soldier fly, Hermetia illucens, as a value-added tool for the management of swine manure. Raleigh (NC): Animal and Poultry Waste Management Center, North Carolina State University; p. 17.

Oba, P. M., P. L. Utterback, C. M. Parsons, M. R. C. de Godoy, and K. S. Swanson. 2019. Chemical composition, true nutrient digestibility, and true metabolizable energy of chicken-based ingredients differing by processing method using the precision-fed cecectomized rooster assay. *J. Anim. Sci.* **97**:998–1009. doi:10.1093/jas/sky461

Oonincx, D. G. A. B., A. van Huis, and J. J. A. van Loon. 2015. Nutrient utilisation by black soldier flies fed with chicken, pig, or cow manure. *J. Insect. Food Feed* **1**:131–139. doi:10.3920/JIFF2014.0023

Overend, G., Y. Luo, L. Henderson, A. E. Douglas, S. A. Davies, and J. A. Dow. 2016. Molecular mechanism and functional significance of acid generation in the Drosophila midgut. *Sci. Rep.* **6**:27242. doi:10.1038/srep27242

Parsons, C. M. 1985. Influence of caecectomy on digestibility of amino acids by roosters fed distillers' dried grains with solubles. *J. Agric. Sci.* **104**:469–472. doi:10.1017/S0021859600044178

Roseland, C. R., M. J. Grodowitz, K. J. Kramer, T. L. Hopkins, and A. B. Broce. 1985. Stabilization of mineralized and sclerotized puparial cuticle in muscid flies. *Insect. Biochem.* **15**:521–528. doi:10.1016/0020-1790(85)90065-4

Selle, P. H., A. J. Cowieson, and V. Ravindran. 2009. Consequences of calcium interactions with phytate and phytase for poultry and pigs. *Livest. Sci.* **124**:126–141. doi:10.1016/j.livsci.2009.01.006

Sheppard, D. C., J. K. Tomberlin, J. A. Joyce, B. C. Kiser, and S. M. Sumner. 2002. Rearing methods for the black soldier fly (Diptera: Stratiomyidae). *J. Med. Entomol.* **39**:695–698. doi:10.1603/0022-2585-39.4.695

Shumo, M. I., M. Osuga, F. M. Khamis, C. M. Tanga, K. K. M. Fiaboe, S. Subramanian, S. Ekesi, A. van Huis, and C. Borgemeister. 2019. The nutritive value of black soldier fly larvae reared on common organic waste streams in Kenya. *Sci. Rep.* **9**:1–13. doi:10.1038/s4159 8-019-46603-z

Sibbald, I. R. 1979. A bioassay for available amino acids and true metabolizable energy in feedstuffs. *Poult. Sci.* **58**:668–673. doi:10.3382/ps.0580668

Spranghers, T., M. Ottoboni, C. Klootwijk, A. Ovyn, S. Deboosere, B. De Meulenaer, J. Michiels, M. Eeckhout, P. De Clercq, and S. De Smet. 2016. Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. *J. Sci. Food Agric.* **97**:2594–2600. doi:10.1002/jsfa.8081

Tomberlin, J. K., P. H. Adler, and H. M. Myers. 2009. Development of the black soldier fly (Diptera: Stratiomyidae) in relation to temperature. *Environ. Entomol.* **38**:930–934. doi:10.1603/022.038.0347

Tomberlin, J. K., D. C. Sheppard, and J. A. Joyce. 2002. Selected life-history traits of the black soldier flies (Diptera: Stratiomyidae) reared on three artificial diets. *Ann. Entomol. Soc. Am.* **95**:379–86. doi:10.1603/0013-8746(2002)095[0379:SLHTOB]2.0.CO;2

Tschirner, M., and A. Simon. 2015. Influence of different growing substrates and processing on the nutrient composition of black soldier fly larvae destined for animal feed. *J. Insect. Food Feed* **1**:249–259. doi:10.3920/JIFF2014.0008

Trailokya, A., A. Srivastava, M. Bhole, and N. Zalte. 2017. Calcium and calcium salts. *J. Assoc. Physicians India* **65**:100–103.

Weaver, M. C. 1998. Calcium in food fortification strategies. *Int. Dairy J.* **8**:443–449. doi:10.1016/S0958-6946(98)00067-3

Wilkinson, S. J., P. H. Selle, M. R. Bedford, and A. J. Cowieson. 2014. Separate feeding of calcium improves performance and ileal nutrient digestibility in broiler chicks. *Anim. Prod. Sci.* **54**:172–178. doi:10.1071/AN12432