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Nutrient and AA digestibility of black soldier fly larvae differing in age using the precision-fed cecectomized rooster assay[1]

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Abstract

Edible insects such as black soldier fly larvae (**BSFL**) are alternative protein sources for animal feeds due to their high-protein content and potential low environmental footprint. However, protein quality and AA content may vary across insect species and age. Our objective was to determine the effects of age on nutrient and AA digestibility of BSFL intended for use in pet foods using the precision-fed cecectomized rooster assay. All animal procedures were approved by the University of Illinois Institutional Animal Care and Use Committee prior to experimentation. Twenty-four cecectomized roosters (four roosters per substrate) were randomly assigned to test substrates [BSFL0 = day 0 (day of hatch); BSFL11 = day 11; BSFL14 = day 14; BSFL18 = day 18; BSFL23 = day 23; BSFL29 = day 29]. 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. DM and OM digestibilities were not different among substrates, but acid-hydrolyzed fat digestibility tended to be greater ($P < 0.10$) for BSFL23 and BSFL29 than BSFL14 and BSFL18. Although all substrates had a high digestibility, BSFL0 and BSFL11 had the lowest ($P < 0.05$) digestibilities for most indispensable and dispensable AA. Digestible indispensable AA score (**DIAAS**)-like values were calculated to determine protein quality according to AAFCO nutrient profiles and NRC recommended allowances for dogs and cats. In general, BSFL18 had the highest, and BSFL11 had the lowest DIAAS-like values for most indispensable AA. Threonine, methionine, and tryptophan were often the first-limiting AA. Our results suggest that BSFL are a high-quality protein and AA source, but that age can affect the AA digestibility and protein quality of this alternative protein source.

Key words: AA digestion, cat, dog, insect meal, pet food, protein source

Introduction

As the world population increases, the production of animal-derived protein sources must as well and is expected to increase more than 75% by 2050 (Alexandratos and Bruinsma, 2012). This increase in demand will cause ecological strain by increasing greenhouse gas emissions, global freshwater stress, and soil acidification (Miglietta et al., 2015). Insects may serve as a potential solution to those problems. For example,

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the average water footprint of mealworms (23 L/g protein) is lower than pork (57 L/g protein), chicken (34 L/g protein), and beef (112 L/g protein). Also, the global warming potential of mealworms per kilogram of edible protein (14 kg of CO_2-eq) is lower than chicken (19 to 37 kg of CO_2-eq), pork (21 to 54 kg of CO_2-eq), and beef (77 to 175 kg of CO_2-eq) and the land use (18 m^2) is lower than that of chicken (41 to 51 m^2), pork (46 to 63 m^2), and beef (142 to 254 m^2) (de Vries and de Boer, 2010; Oonincx and de Boer, 2012; Miglietta et al., 2015). Therefore, insects may be considered as a more sustainable protein source compared with traditional animal-derived protein sources for human consumption.

Black soldier fly larvae (**BSFL**; *Hermetia illucens*) have attracted substantial attention worldwide in recent years because they may serve as an alternative protein source for pet foods given their many economic, nutritional, and environmental advantages. BSFL have several advantages over other insect species, including the number of growth cycles possible per year, the potential for vertical farming, and protein yield (Koutsos et al., 2019). One of their benefits may be due to the variety of digestive enzymes (α-amylase, lipase, and proteases) they secrete by the salivary gland and gastrointestinal tract, leading to more effective digestion and nutrient accumulation than house fly larvae (Kim et al., 2011). For this reason, during the larval stage, BSFL perform well on a variety of organic materials (animal waste and plant material). Despite their ability to grow on waste materials, in the United States and Europe, they must be fed feed-grade ingredients and adhere to standard safe feeding conditions in order to be fed to livestock or companion animals. The restriction to approved, feed-grade materials are intended to ensure production of safe larvae, which could accumulate pathogens, heavy metals, or other toxins from less-regulated inputs (AAFCO, 2016).

The life cycle of BSFL ranges between several weeks to several months depending on the environmental temperature and humidity and quality of diet (Veldkamp et al., 2012; Wang and Shelomi, 2017). Female BSFL lay over 500 eggs in a dry environment. After 4 to 5 d, the eggs hatch and start to consume their diet. Two to 4 wk later, the larvae reach the prepupal stage, stop feeding, and move to sheltered spaces for pupa development (Sheppard et al., 2002; Tomberlin and Sheppard, 2002; Liu et al., 2017). In most previous studies, the prepupal stage of BSFL has been evaluated for nutritional composition analysis for poultry or swine diets (Kroeckel et al., 2012; Cullere et al., 2016; Barragan-Fonseca et al., 2017). To our knowledge, limited scientific data have shown how different harvest age affects BSFL nutrient composition and its bioavailability for animal feeds (Liu et al., 2017). Therefore, it is necessary to evaluate the protein quality of various ages of BSFL for animal feed.

The cecectomized rooster assay has been used as a model for measuring nutrient and AA digestibility of feed ingredients of pet foods because results were shown to be similar to that of ileal-cannulated dogs (Johnson et al., 1998). Like ileal-cannulated animals, cecectomy allows for digestibility estimates to be made with minimal interference from the bacterial fermentation of proteins in the hindgut. Also, the cecectomized rooster assay is less expensive, time-consuming, and labor-intensive than other types of assays (Johnson et al., 1998; Faber et al., 2010; Kerr et al., 2014; Oba et al., 2019). Given these advantages, the cecectomized rooster assay is often a preferred model to evaluate the protein quality of novel ingredients for dogs and cats.

The objective of this study was to determine the effects of the age on nutrient and AA digestibility of BSFL intended for use in pet foods using the precision-fed cecectomized rooster assay.

MATERIALS AND METHODS

Substrates

BSFL were harvested at six different ages and used for testing in this study. Because the nutritional value of the diet affects BSFL growth rate, the ages (days after hatch) and weights of BSFL studied herein are provided: day 0 (BSFL0): 0.02 mg; day 11 (BSFL11): 0.08 mg; day 14 (BSFL14): 0.14 mg; day 18 (BSFL18): 0.14 mg; day 23 (BSFL23): 0.19 mg; day 29 (BSFL29): 0.21 mg. Industry standard rearing conditions were maintained (Sheppard et al., 2002). All insects were fed a commercial layer ration until 11 d of age. From day 11, they were fed a combination of distiller's dried grains with solubles from a distillery, bakery by-product meal, and calcium chloride. At the time of collection for this trial, larvae were washed, frozen, and shipped to the University of Illinois for further processing and preparation. All ages of BSFL were lyophilized and ground through a 2-mm screen with dry ice to allow for proper grinding before 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 nutrient 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, 24 cecectomized roosters were randomly assigned to the test substrates (four 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. Macronutrient 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 OM according to AOAC (2006). Nitrogen and CP were determined using a Leco Nitrogen/Protein Determinator (Model FP-2000, Leco Corporation, St. Joseph, MI) according to the AOAC (2006; method 982.30E). Fat concentrations were measured by acid hydrolysis according to the AACC (1983) followed by diethyl ether extraction (Budde, 1952). GE was measured using a bomb calorimeter (Model 1261; Parr Instrument Co., Moline, IL). AAs were measured at the University of Missouri Experiment Station Chemical Laboratories (Columbia, MO) according to the AOAC (2006; method 982.30E).

Digestible Indispensable AA Score (DIAAS)-Like Calculations

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 AAFCO nutrient profiles (AAFCO, 2019) 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 FAO (2011): DIAAS-like % = 100 × [(mg of digestible dietary indispensable AA in 1g 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 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 LS 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. In regards to chemical composition, OM was highest in BSFL0 (94.8% dry matter basis [DMB]) and was steadily reduced with age. CP, acid-hydrolyzed fat (**AHF**), and GE were variable. CP was highest in BSFL0 (57.2% DMB), was reduced to half that level by day 11 (26.8% DMB), and then steadily increased until day 29 (40.3% DMB). AHF was highest at BSFL14 (39.7% DMB) and BSFL11 (38.0%), and lower for the other ages (30.2%

Table 1. Chemical composition and macronutrient digestibilities of BSFL differing in age using the precision-fed cecectomized rooster assay

Item	BSFL0[1]	BSFL11	BSFL14	BSFL18	BSFL23	BSFL29	SEM	P-value
Chemical composition								
DM, %	96.02	93.97	93.93	93.74	89.26	91.96	—	—
OM, % DM	94.82	91.38	91.49	89.65	86.54	85.87	—	—
CP, % DM	57.20	26.77	31.31	37.72	39.74	40.34	—	—
AHF, % DM	31.44	37.98	39.74	34.45	30.20	32.45	—	—
GE, kcal/g DM	6.29	5.95	6.10	5.90	5.40	5.57	—	—
Nutrient digestibility								
DM, %	54.61	63.24	58.43	55.73	61.11	58.26	1.312	0.4386
OM, %	64.26	75.57	69.85	68.49	71.64	67.64	1.299	0.1952
AHF, %	83.58[xy]	82.00[xy]	80.27[y]	80.77[y]	88.26[x]	86.36[x]	0.904	0.0401

[1]BSFL0, day 0, day of hatch of black soldier fly larvae; BSFL11, day 11 of age of black soldier fly larvae; BSFL14, day 14 of age of black soldier fly larvae; BSFL18, day 18 of age of black soldier fly larvae; BSFL23, day 23 of age of black soldier fly larvae; BSFL29, day 29 of age of black soldier fly larvae; AHF, acid-hydrolyzed fat.

[x,y]Within a row, means lacking a common superscript differ ($P < 0.10$); *n,* 4 roosters per treatment.

Table 2. Indispensable and dispensable AA concentrations (% DM) of BSFL differing in age

Item	BSFL0[1]	BSFL11	BSFL14	BSFL18	BSFL23	BSFL29
Indispensable AA						
Arginine	3.18	1.22	1.46	1.82	1.86	2.05
Histidine	1.45	0.78	0.94	1.19	1.18	1.29
Isoleucine	2.49	1.10	1.35	1.67	1.79	1.82
Leucine	3.71	1.68	2.02	2.58	2.76	2.80
Lysine	3.81	1.65	2.12	2.67	2.67	2.71
Methionine	0.93	0.40	0.48	0.67	0.70	0.74
Phenylalanine	1.95	0.97	1.22	1.67	1.76	1.73
Threonine	2.10	1.00	1.23	1.52	1.54	1.57
Tryptophan	0.57	0.26	0.40	0.57	0.58	0.50
Valine	3.13	1.58	2.10	2.91	3.03	3.23
Selected dispensable AA						
Alanine	3.71	1.96	2.24	2.71	2.56	2.35
Aspartic acid	4.32	1.88	2.49	3.39	3.52	3.63
Cysteine	0.60	0.36	0.35	0.36	0.31	0.28
Glutamic acid	6.38	2.95	3.30	3.80	3.70	3.63
Glycine	2.87	1.28	1.59	1.97	2.30	2.43
Proline	2.87	1.73	1.97	2.39	2.44	2.30
Serine	2.21	1.04	1.28	1.55	1.58	1.62
Tyrosine	2.14	1.20	1.79	2.48	2.63	2.64
Taurine	0.05	0.10	0.09	0.08	0.08	0.07

[1]BSFL0, day 0, day of hatch of black soldier fly larvae; BSFL11, day 11 of age of black soldier fly larvae; BSFL14, day 14 of age of black soldier fly larvae; BSFL18, day 18 of age of black soldier fly larvae; BSFL23, day 23 of age of black soldier fly larvae; BSFL29, day 29 of age of black soldier fly larvae.

to 34.5% DMB). The GE values ranged from 5.40 (BSFL23) to 6.29 kcal/g DM (BSFL0). Concentrations of indispensable and dispensable AA are presented in Table 2. Not surprisingly, AA patterns were similar to that of CP. The BSFL0 age showed higher AA concentrations than other stages of BSFL except for tryptophan, valine, tyrosine, and taurine. On the other hand, BSFL11 had lower indispensable AA concentrations than other BSFL ingredients.

Cecectomized Rooster Assay

Macronutrient digestibilities of tested BSFL ingredients are presented in Table 1. All animals remained healthy during the study. There were no significant differences in DM and OM digestibility among substrates, but AHF tended to be greater ($P < 0.10$) for BSFL23 and BSFL29 than BSFL14 and BSFL18.

AA digestibilities data are presented in Table 3, with many differences being observed. Of the indispensable AA, day 0 BSFL had lower ($P < 0.05$) methionine and phenylalanine digestibilities than those harvested at day 23 of age. Day 11 BSFL had a lower ($P < 0.05$) tryptophan digestibility than day 18 and 23 BSFL. Day 11 BSFL also had a lower ($P < 0.05$) methionine digestibility than day 23 BSFL. Day 29 BSFL had a lower ($P < 0.05$) leucine digestibility than day 18 BSFL. Also, days 0 and 29 BSFL tended to have a lower ($P < 0.10$) histidine digestibility than days 11, 14, 18, and 23 BSFL. Of the dispensable AA, day 29 BSFL had a lower ($P < 0.05$) alanine digestibility than all other BSFL ages except for day 0. Finally, day 0 BSFL had lower ($P < 0.05$) tyrosine digestibility than day 18 BSFL.

DIAAS-Like Calculations

DIAAS-like values for growing puppies and kittens are presented in Tables 4 and 5, respectively. DIAAS-like values for adult dogs and cats at maintenance are presented in Tables 6 and 7, respectively. The first limiting AA based on DIAAS-like calculations from AAFCO (2019) nutrient profiles and NRC (2006) recommended allowances are listed in Tables 8 and 9, respectively.

Based on the AAFCO recommended allowances for growing puppies, all BSFL ingredients had some DIAAS-like values below 100%. BSFL18, 23 and 29 had the most DIAAS-like values over 100% (all except threonine) followed by BSFL14, BSFL0, and BSFL11. Using the NRC recommended allowances for growing puppies, BSFL18 had no DIAAS-like values below 100%, and BSFL23 and 29 had only DIAAS-like values below 100% for threonine followed by BSFL14, BSFL11, and BSFL0. According to the AAFCO and NRC recommended allowances for growing kittens, all BSFL ingredients had only DIAAS-like values below 100% for methionine, and all other indispensable AA values were over 100%.

Based on the AAFCO recommended allowances for adult dogs, all BSFL ingredients had DIAAS-like values over 100% except methionine (and tryptophan for BSFL11). Using the NRC recommended allowances for an adult dog, BSFL18 had DIAAS-like values below 100% for leucine, methionine, phenylalanine, and threonine followed by BSFL23, BSFL29, BSFL11, and BSFL0. According to the AAFCO and NRC recommended allowances for adult cats, all BSFL ingredients had sufficient AA concentration for adult cats.

The first-limiting AA based on DIAAS-like values from AAFCO (2019) nutrient profiles for dogs and cats (growth and reproduction; adults at maintenance) are provided in Table 8. For growing and reproducing dogs, threonine was the first limiting AA for all ages of BSFL ingredients except for BSFL14 that was limited by tryptophan. All of those values were <100, suggesting insufficiency if a diet was formulated with only that protein source and at an inclusion level to meet the nutrient profile. Based on DIAAS-like values from AAFCO (2019) nutrient profiles for adult dogs at maintenance, methionine was the first limiting AA for all ages of BSFL. Based on DIAAS-like values from AAFCO (2019) nutrient profiles for growing and reproducing cats, methionine was the first limiting for all ages of BSFL. Based on

Table 3. AA digestibilities (%) of BSFL differing in age using the precision-fed cecectomized rooster assay

Item	BSFL0[1]	BSFL11	BSFL14	BSFL18	BSFL23	BSFL29	SEM	P-value
Indispensable AA								
Arginine	90.77	93.42	94.05	94.09	95.52	90.64	0.647	0.1623
Histidine	82.99[y]	89.58[x]	89.09[x]	90.91[x]	87.82[x]	82.87[y]	0.959	0.0301
Isoleucine	86.97	89.29	90.67	92.06	92.52	86.72	0.718	0.0504
Leucine	87.02[ab]	91.22[ab]	92.04[ab]	93.58[a]	92.90[ab]	85.99[b]	0.852	0.0186
Lysine	87.75	89.12	91.03	90.49	91.36	90.16	0.721	0.7619
Methionine	89.43[b]	89.15[b]	90.79[ab]	92.63[ab]	96.04[a]	92.78[ab]	0.670	0.0108
Phenylalanine	85.81[b]	88.46[ab]	90.05[ab]	92.34[ab]	93.98[a]	90.18[ab]	0.764	0.0165
Threonine	87.37	87.01	88.55	92.16	92.67	88.08	0.921	0.3176
Tryptophan	89.98[ab]	89.50[b]	92.09[ab]	94.43[a]	94.46[a]	92.56[ab]	0.551	0.0109
Valine	80.88	79.75	83.79	85.29	81.64	75.34	1.117	0.1383
Selected dispensable AA								
Alanine	89.99[ab]	92.09[a]	92.53[a]	93.95[a]	91.46[a]	82.15[b]	1.024	0.0023
Aspartic acid	89.88	88.02	90.22	92.55	94.28	91.00	0.686	0.1134
Cysteine	79.22	81.44	78.38	79.00	87.40	81.89	1.809	0.7679
Glutamic acid	90.42	89.28	88.41	89.85	92.08	88.01	0.784	0.7484
Glycine	76.29	82.56	70.27	78.37	84.96	73.72	2.259	0.4602
Proline	85.82	88.57	89.25	91.39	90.18	83.04	0.957	0.1039
Serine	86.71	86.87	89.51	90.42	91.12	81.47	1.111	0.1112
Tyrosine	85.06[b]	89.31[ab]	91.03[ab]	93.39[a]	91.77[ab]	86.94[ab]	0.807	0.0097

[1]BSFL0, day 0, day of hatch of black soldier fly larvae; BSFL11, day 11 of age of black soldier fly larvae; BSFL14, day 14 of age of black soldier fly larvae; BSFL18, day 18 of age of black soldier fly larvae; BSFL23, day 23 of age of black soldier fly larvae; BSFL29, day 29 of age of black soldier fly larvae.

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

[x,y]Within a row, means lacking a common superscript differ ($P < 0.10$); n, 4 roosters per treatment.

Table 4. Digestible indispensable AA scores (DIAAS)—like values[1] of BSFL differing in age for growing puppies[2]

	AAFCO (2019)						NRC (2006)					
Item	**BSFL0[3]**	**BSFL11**	**BSFL14**	**BSFL18**	**BSFL23**	**BSFL29**	**BSFL0**	**BSFL11**	**BSFL14**	**BSFL18**	**BSFL23**	**BSFL29**
Indispensable AA												
Arginine	113.54^{a}	95.78^{c}	98.68^{bc}	102.14^{bc}	100.59^{bc}	103.65^{b}	143.72^{a}	121.24^{c}	124.91^{bc}	129.30^{bc}	127.33^{bc}	131.20^{b}
Histidine	107.58	133.46	136.78	146.66	133.34	135.51	121.37^{b}	150.57^{a}	154.32^{a}	165.46^{a}	150.44^{a}	152.89^{a}
Isoleucine	119.98^{bc}	116.25^{c}	123.89^{abc}	129.16^{ab}	132.06^{a}	123.99^{abc}	131.05^{bc}	126.98^{c}	135.33^{abc}	141.08^{ab}	144.25^{a}	135.43^{abc}
Leucine	98.45^{c}	99.83^{c}	103.57^{bc}	111.63^{ab}	112.54^{a}	104.11^{abc}	98.45^{c}	99.83^{c}	103.57^{bc}	111.63^{ab}	112.54^{a}	104.11^{abc}
Lysine	146.13^{bc}	137.31^{c}	154.09^{ab}	160.12^{a}	153.45^{ab}	151.42^{ab}	149.45^{bc}	140.43^{c}	157.59^{ab}	163.76^{a}	156.94^{ab}	154.86^{ab}
Methionine	93.47^{b}	85.63^{c}	89.48^{bc}	105.76^{a}	108.75^{a}	109.41^{a}	99.14^{b}	90.81^{c}	94.90^{bc}	112.17^{a}	115.34^{a}	116.04^{a}
Phenylalanine	79.30^{e}	86.88^{d}	95.12^{c}	110.82^{ab}	112.83^{a}	104.84^{b}	101.26^{e}	110.93^{d}	121.46^{c}	141.51^{ab}	144.08^{a}	133.87^{b}
Threonine	69.39^{c}	70.30^{bc}	75.26^{abc}	80.34^{a}	77.70^{ab}	74.17^{abc}	89.10^{c}	90.27^{bc}	96.63^{abc}	103.16^{a}	99.76^{ab}	95.23^{abc}
Tryptophan	100.88^{c}	97.77^{c}	132.35^{b}	160.53^{a}	155.10^{a}	129.06^{b}	87.72^{c}	85.02^{c}	115.89^{b}	139.59^{a}	134.87^{a}	112.23^{b}
Valine	146.44^{c}	155.73^{c}	185.96^{b}	217.72^{a}	205.97^{ab}	199.61^{ab}	146.44^{c}	155.73^{c}	185.96^{b}	217.72^{a}	205.97^{ab}	199.61^{ab}

[1]DIAAS-like values were calculated from the digestibility of AAs in cecectomized roosters.
[2]DIAAS-like values were calculated using the AAFCO (2019) nutrient profiles of AAs for growth and reproduction of dogs, and NRC (2006) recommended allowances of AAs for growing puppies (4 to 14 wk of age).
[3]BSFL0, day 0, day of hatch of black soldier fly larvae; BSFL11, day 11 of age of black soldier fly larvae; BSFL14, day 14 of age of black soldier fly larvae; BSFL18, day 18 of age of black soldier fly larvae, BSFL23, day 23 of age of black soldier fly larvae; BSFL29, day 29 of age of black soldier fly larvae. SEM values for AAFCO (2019) nutrient profile data: arginine, 1.307; histidine, 2.704; isoleucine, 1.336; leucine, 1.327; lysine, 1.858; methionine, 2.052; phenylalanine, 2.633; threonine, 1.034; tryptophan, 5.023; valine, 5.877. SEM values for NRC (2006) recommended allowance data: arginine, 1.654; histidine, 3.051; isoleucine, 1.459; leucine, 1.327; lysine, 1.900; methionine, 2.177; phenylalanine, 3.362; threonine, 1.327; tryptophan, 4.368; valine, 5.877.
[a-e]Within a row, means lacking a common superscript differ ($P<0.05$); n, 4 roosters per treatment.

Table 5. Digestible indispensable AA scores (DIAAS)—like values[1] of BSFL differing in age for growing kittens[2]

	AAFCO (2019)						NRC (2006)					
Item	**BSFL0[3]**	**BSFL11**	**BSFL14**	**BSFL18**	**BSFL23**	**BSFL29**	**BSFL0**	**BSFL11**	**BSFL14**	**BSFL18**	**BSFL23**	**BSFL29**
Indispensable AA												
Arginine	122.08^{a}	102.99^{c}	106.11^{bc}	109.83^{bc}	108.16^{bc}	111.45^{b}	131.41^{a}	110.86^{c}	114.21^{bc}	118.22^{bc}	116.43^{bc}	119.96^{b}
Histidine	191.25^{b}	237.26^{a}	243.16^{a}	260.73^{a}	237.05^{a}	240.91^{a}	159.38^{b}	197.71^{a}	202.64^{a}	217.27^{a}	197.55^{a}	200.76^{a}
Isoleucine	202.82^{bc}	196.52^{c}	209.43^{abc}	218.33^{ab}	223.25^{a}	209.60^{abc}	175.27^{bc}	169.83^{c}	180.99^{abc}	188.68^{ab}	192.93^{a}	181.13^{abc}
Leucine	132.29^{c}	134.15^{c}	139.17^{bc}	150.01^{ab}	151.22^{a}	139.90^{abc}	110.24^{c}	111.79^{c}	115.97^{bc}	125.01^{ab}	126.02^{a}	116.58^{abc}
Lysine	146.13^{bc}	137.31^{c}	154.09^{ab}	160.12^{a}	153.45^{ab}	151.42^{ab}	171.91^{bc}	161.54^{c}	181.28^{ab}	188.38^{a}	180.53^{ab}	178.14^{ab}
Methionine	70.35^{b}	64.45^{c}	67.35^{bc}	79.61^{a}	81.86^{a}	82.35^{a}	82.61^{b}	75.68^{c}	79.09^{bc}	93.48^{a}	96.12^{a}	96.70^{a}
Phenylalanine	168.77^{e}	184.89^{d}	202.43^{c}	235.85^{ab}	240.13^{a}	223.12^{b}	146.27^{e}	160.24^{d}	175.44^{c}	204.40^{ab}	208.11^{a}	193.37^{b}
Threonine	131.82^{c}	133.55^{bc}	142.96^{abc}	152.62^{a}	147.59^{ab}	140.88^{abc}	123.37^{c}	124.99^{bc}	133.79^{abc}	142.83^{a}	138.13^{ab}	131.85^{abc}
Tryptophan	107.60^{c}	104.29^{c}	141.18^{b}	171.23^{a}	165.44^{a}	137.67^{b}	140.10^{c}	135.80^{c}	183.82^{b}	222.95^{a}	215.41^{a}	179.25^{b}
Valine	207.46^{c}	220.61^{c}	263.45^{b}	308.43^{a}	291.79^{ab}	282.78^{ab}	172.88^{c}	183.74^{c}	219.54^{b}	257.03^{a}	243.16^{ab}	235.65^{ab}

[1]DIAAS-like values were calculated from the digestibility of AAs in cecectomized roosters.
[2]DIAAS-like values were calculated using the AAFCO (2019) nutrient profiles of AAs for growth and reproduction of cats, and NRC (2006) recommended allowances of AAs for growing kittens.
[3]BSFL0, day 0, day of hatch of black soldier fly larvae; BSFL11, day 11 of age of black soldier fly larvae; BSFL14, day 14 of age of black soldier fly larvae; BSFL18, day 18 of age of black soldier fly larvae; BSFL23; day 23 of age of black soldier fly larvae; BSFL29, day 29 of age of black soldier fly larvae. SEM values for AAFCO (2019) nutrient profile data: arginine, 1.405; histidine, 4.808; isoleucine, 2.258; leucine, 1.783; lysine, 1.858; methionine, 1.545; phenylalanine, 5.603; threonine, 1.964; tryptophan, 5.358; valine, 8.326. SEM values for NRC (2006) recommended allowance data: arginine, 1.512; histidine, 4.006; isoleucine, 1.952; leucine, 1.486; lysine, 2.186; methionine, 1.814; phenylalanine, 4.856; threonine, 1.838; tryptophan, 6.977; valine, 6.934.
[a–e]Within a row, means lacking a common superscript differ ($P < 0.05$); *n,* 4 roosters per treatment.

DIAAS-like values from AAFCO (2019) nutrient profiles for adult cats at maintenance, threonine (BSFL0) and arginine (BSFL11; BSFL14, BSFL18; BSFL23 and BSFL29) were the first limiting AA, but all were >100.

The first-limiting AA based on DIAAS-like values from NRC (2006) recommended allowances for growing puppies (4 to 14 wk of age), growing kittens, and adult dogs and cats at maintenance are provided in Table 9. For growing puppies, tryptophan (BSFL0; BSFL11), methionine (BSFL14), and threonine (BSFL18; BSFL23 and BSFL29) were the first limiting AA, but BSFL18 and BSFL23 were >100. Based on DIAAS-like values from NRC (2006) recommended allowances for adult dogs at maintenance, methionine was the first limiting AA for all ages of BSFL ingredients. All of those values were <100. Based on DIAAS-like values from NRC (2006) recommended allowances for growing kittens, methionine was the first limiting AA for all ages of BSFL ingredients. Based on DIAAS-like values from NRC (2006) recommended allowances for adult cats, arginine (BSFL11; BSFL14; BSFL18 and BSFL23), and leucine (BSFL0 and BSFL29) were the first limiting AA, with all values being >100.

Table 6. Digestible indispensable AA scores (DIAAS)—like values[1] of BSFL differing in age for adult dogs[2]

	AAFCO (2019)						NRC (2006)					
Item	**BSFL0**[3]	**BSFL11**	**BSFL14**	**BSFL18**	**BSFL23**	**BSFL29**	**BSFL0**	**BSFL11**	**BSFL14**	**BSFL18**	**BSFL23**	**BSFL29**
Indispensable AA												
Arginine	178.10^{a}	150.24^{c}	154.79^{bc}	160.23^{bc}	157.79^{bc}	162.85^{b}	144.17^{a}	121.62^{c}	125.31^{bc}	129.71^{bc}	127.74^{bc}	131.61^{b}
Histidine	199.31^{b}	247.25^{a}	253.40^{a}	271.70^{a}	247.04^{a}	251.06^{a}	110.73^{b}	137.36^{a}	140.78^{a}	150.95^{a}	137.24^{a}	139.48^{a}
Isoleucine	179.33^{bc}	173.76^{c}	185.18^{abc}	193.05^{ab}	197.40^{a}	185.33^{abc}	99.63^{bc}	96.53^{c}	102.88^{abc}	107.25^{ab}	109.67^{a}	102.96^{abc}
Leucine	149.41^{c}	151.51^{c}	157.18^{bc}	169.42^{ab}	170.79^{a}	158.00^{abc}	83.01^{c}	84.17^{c}	87.32^{bc}	94.12^{ab}	94.88^{a}	87.78^{abc}
Lysine	167.00^{bc}	156.92^{c}	176.10^{ab}	182.99^{a}	175.38^{ab}	173.05^{ab}	167.00^{bc}	156.92^{c}	176.10^{ab}	182.99^{a}	175.38^{ab}	173.05^{ab}
Methionine	79.31^{b}	72.65^{c}	75.92^{bc}	89.74^{a}	92.28^{a}	92.84^{a}	44.06^{b}	40.36^{c}	42.18^{bc}	49.85^{a}	51.26^{a}	51.58^{a}
Phenylalanine	117.01^{e}	128.19^{d}	140.35^{c}	163.52^{ab}	166.49^{a}	154.70^{b}	65.01^{e}	71.22^{d}	77.97^{c}	90.85^{ab}	92.49^{a}	85.94^{b}
Threonine	120.28^{c}	121.86^{bc}	130.45^{abc}	139.26^{a}	134.68^{ab}	128.56^{abc}	74.59^{c}	75.57^{bc}	80.90^{abc}	86.36^{a}	83.52^{ab}	79.72^{abc}
Tryptophan	100.88^{c}	97.77^{c}	132.35^{b}	160.52^{a}	155.10^{a}	129.06^{b}	64.05^{c}	62.08^{c}	84.03^{b}	101.92^{a}	98.47^{a}	81.94^{b}
Valine	162.58^{c}	172.89^{c}	206.46^{b}	241.71^{a}	228.67^{ab}	221.61^{ab}	90.32^{c}	96.05^{c}	114.70^{b}	134.28^{a}	127.04^{ab}	123.12^{ab}

[1]DIAAS-like values were calculated from the digestibility of AAs in cecectomized roosters.
[2]DIAAS-like values were calculated using the AAFCO (2019) nutrient profiles and NRC (2006) recommended allowances of AAs for adult dogs at maintenance.
[3]BSFL0, day 0, day of hatch of black soldier fly larvae; BSFL11, day 11 of age of black soldier fly larvae; BSFL14, day 14 of age of black soldier fly larvae; BSFL18, day 18 of age of black soldier fly larvae; BSFL23, day 23 of age of black soldier fly larvae; BSFL29, day 29 of age of black soldier fly larvae. SEM values for AAFCO (2019) nutrient profile data: arginine, 2.050; histidine, 5.010; isoleucine, 1.997; leucine, 2.014; lysine, 2.123; methionine, 1.741; phenylalanine, 3.884; threonine, 1.792; tryptophan, 5.023; valine, 6.525. SEM values for NRC (2006) recommended allowance data: arginine, 1.659; histidine, 2.783; isoleucine, 1.109; leucine, 1.119; lysine, 2.123; methionine 0.967; phenylalanine, 2.158; threonine, 1.111; tryptophan, 3.189; valine, 3.625.
[a–e]Within a row, means lacking a common superscript differ ($P < 0.05$); *n*, 4 roosters per treatment.

Table 7. Digestible indispensable AA scores (DIAAS)—like values[1] of BSFL differing in age for adult cats[2]

	AAFCO (2019)						NRC (2006)					
Item	**BSFL0**[3]	**BSFL11**	**BSFL14**	**BSFL18**	**BSFL23**	**BSFL29**	**BSFL0**	**BSFL11**	**BSFL14**	**BSFL18**	**BSFL23**	**BSFL29**
Indispensable AA												
Arginine	126.15^{a}	106.42^{c}	109.65^{bc}	113.49^{bc}	111.77^{bc}	115.16^{b}	131.07^{a}	110.57^{c}	113.92^{bc}	117.92^{bc}	116.12^{bc}	119.65^{b}
Histidine	176.45^{b}	218.89^{a}	224.34^{a}	240.54^{a}	218.70^{a}	222.26^{a}	161.83^{b}	200.76^{a}	205.75^{a}	220.61^{a}	200.58^{a}	203.85^{a}
Isoleucine	189.30^{bc}	183.42^{c}	195.47^{abc}	203.78^{ab}	208.37^{a}	195.62^{abc}	176.09^{bc}	170.62^{c}	181.83^{abc}	189.56^{ab}	193.83^{a}	181.98^{abc}
Leucine	118.35^{c}	120.01^{c}	124.50^{bc}	134.20^{ab}	135.29^{a}	125.15^{abc}	110.67^{c}	112.23^{c}	116.43^{bc}	125.50^{ab}	126.51^{a}	117.04^{abc}
Lysine	183.10^{bc}	172.05^{c}	193.07^{ab}	200.63^{a}	192.28^{ab}	189.73^{ab}	343.82^{bc}	323.07^{c}	362.56^{ab}	376.75^{a}	361.07^{ab}	356.27^{ab}
Methionine	189.02^{b}	173.15^{c}	180.95^{bc}	213.88^{a}	219.92^{a}	221.26^{a}	171.06^{b}	156.70^{c}	163.76^{bc}	193.55^{a}	199.03^{a}	200.23^{a}
Phenylalanine	190.15^{e}	208.31^{d}	228.07^{c}	265.72^{ab}	270.54^{a}	251.38^{b}	146.27^{e}	160.24^{d}	175.44^{c}	204.40^{ab}	208.11^{a}	193.37^{b}
Threonine	114.24^{c}	115.74^{bc}	123.90^{abc}	132.27^{a}	127.91^{ab}	122.10^{abc}	123.37^{c}	124.99^{bc}	133.79^{abc}	142.83^{a}	138.13^{ab}	131.85^{abc}
Tryptophan	145.71^{c}	141.23^{c}	191.78^{b}	231.87^{a}	224.03^{a}	186.42^{b}	137.95^{c}	133.71^{c}	181.00^{b}	219.52^{a}	212.10^{a}	176.49^{b}
Valine	185.60^{c}	197.36^{c}	235.68^{b}	275.93^{a}	261.04^{ab}	252.98^{ab}	173.56^{c}	184.56^{c}	220.40^{b}	258.04^{a}	244.11^{ab}	236.58^{ab}

[1]DIAAS-like values were calculated from the digestibility of AAs in cecectomized roosters.
[2]DIAAS-like values were calculated using the AAFCO (2019) nutrient profiles and NRC (2006) recommended allowances of AAs for adult cats at maintenance.
[3]BSFL0, day 0, day of hatch of black soldier fly larvae; BSFL11, day 11 of age of black soldier fly larvae; BSFL14, day 14 of age of black soldier fly larvae; BSFL18, day 18 of age of black soldier fly larvae; BSFL23, day 23 of age of black soldier fly larvae; BSFL29, day 29 of age of black soldier fly larvae. SEM values for AAFCO (2019) nutrient profile data: arginine, 1.452; histidine, 4.436; isoleucine, 2.108; leucine, 1.595; lysine, 2.328; methionine, 4.150; phenylalanine, 6.312; threonine, 1.702; tryptophan, 7.256; valine, 7.449. SEM values for NRC (2006) recommended allowance data: arginine, 1.508; histidine, 4.068; isoleucine, 1.961; leucine, 1.492; lysine, 4.371; methionine, 3.756; phenylalanine, 4.856; threonine, 1.838; tryptophan, 6.870; valine, 6.966.
[a–e]Within a row, means lacking a common superscript differ ($P < 0.05$); *n*, 4 roosters per treatment.

Discussion

The demand for animal-derived protein sources will continue to increase in coming years because of the growing human population and rising living standards in developing countries (FAO, 2009). This demand will increase the competition for protein sources in human foods, pet foods, and livestock feeds (Bosch et al., 2014). BSFL may be used to address this problem. Compared with other insects, BSFL may provide a higher number of growth cycles per year, a greater potential for vertical farming, and total protein yield (Koutsos et al., 2019). In order to produce high-quality BSFL protein, the quality of food fed to BSFL is important as it correlates with their larval development time and nutrient composition. According to Oonincx et al. (2015), larval development time was increased when BSFL were fed a low-protein diet (over 5 wk) compared with a high-protein, high-fat diet (3 wk). Moreover, the EE and ash content of the substrates fed (chicken feed, digestate, vegetable, and restaurant waste) was highly correlated with EE (r = 0.942) and ash (r = 0.954) content of BSFL in the prepupa stage (Spranghers et al., 2017). Many other factors also influence

Table 8. First-limiting AA based on digestible indispensable AA scores (DIAAS)—like values[1] of BSFL differing in age from AAFCO (2019) nutrient profiles[2]

	Growth and reproduction		Adults	
Item[3]	Dogs	Cats	Dogs	Cats
BSFL0	69 (Thr)	70 (Met)	79 (Met)	114 (Thr)
BSFL11	70 (Thr)	64 (Met)	73 (Met)	106 (Arg)
BSFL14	75 (Trp)	67 (Met)	76 (Met)	110 (Arg)
BSFL18	80 (Thr)	80 (Met)	90 (Met)	113 (Arg)
BSFL23	78 (Thr)	82 (Met)	92 (Met)	112 (Arg)
BSFL29	74 (Thr)	82 (Met)	93 (Met)	115 (Arg)

[1]DIAAS-like values were calculated from the digestibility of AAs in cecectomized roosters.
[2]DIAAS-like values were calculated using the AAFCO (2019) nutrient profiles of AAs for dogs and cats.
[3]BSFL0, day 0, day of hatch of black soldier fly larvae; BSFL11, day 11 of age of black soldier fly larvae; BSFL14, day 14 of age of black soldier fly larvae; BSFL18, day 18 of age of black soldier fly larvae; BSFL23, day 23 of age of black soldier fly larvae; BSFL29, day 29 of age of black soldier fly larvae.

Table 9. First-limiting AA based on digestible indispensable AA scores (DIAAS)—like values[1] of BSFL differing in age from NRC (2006) recommended allowances[2]

			Adult	
Item[3]	Puppies, (4 to 14 wk of age)	Kittens	Dogs	Cats
BSFL0	87 (Trp)	83 (Met)	44 (Met)	111 (Leu)
BSFL11	85 (Trp)	76 (Met)	40 (Met)	111 (Arg)
BSFL14	95 (Met)	79 (Met)	42 (Met)	114 (Arg)
BSFL18	103 (Thr)	93 (Met)	50 (Met)	118 (Arg)
BSFL23	100 (Thr)	96 (Met)	51 (Met)	117 (Arg)
BSFL29	95 (Thr)	97 (Met)	52 (Met)	117 (Leu)

[1]DIAAS-like values were calculated from the digestibility of AAs in cecectomized roosters.
[2]DIAAS-like values were calculated using the NRC (2006) recommended allowances of AAs for dogs and cats.
[3]BSFL0, day 0, day of hatch of black soldier fly larvae; BSFL11, day 11 of age of black soldier fly larvae; BSFL14, day 14 of age of black soldier fly larvae; BSFL18, day 18 of age of black soldier fly larvae; BSFL23, day 23 of age of black soldier fly larvae; BSFL29, day 29 of age of black soldier fly larvae.

the nutrient composition of BSFL, including sex (Sönmez and Gülel, 2008), stage of development (McClement et al., 2003), and environmental factors (temperature and humidity) (Sönmez and Gülel, 2008; Nedvěd and Kalushkov, 2012). Because these factors may have such large effects, research is needed to test the protein quality of different ages of BSFL.

In the current study, day 0 BSFL had the highest CP content, and from days 11 to 29, CP content linearly increased because BSFL tends to store nutrients in their body for the adult stage. Also, ash content linearly increased from days 0 to 29 due to the development of their exoskeleton. BSFL AHF composition varied considerably from days 0 to 29, but was typically changing over time in a manner that was opposite to that of CP. This pattern of CP and AHF deposition has been reported previously. According to Liu et al. (2017), the CP content of BSFL increased after hatching, and then it slowly decreased until day 14, while it increased in pupal and adult stages. The diet fed after day 11 for BSFL in the present study may have affected CP content during the larval stage. The previous study reported that BSFL CP content was affected by CP content of test substrates. The CP content of BSFL increased when fed a low-protein diet (10% CP) compared with a high-protein diet (17% CP) (Barragan-Fonseca et al., 2019), and other studies also showed that CP content of BSFL decreased with increased CP content in the BSFL diet (Tschirner and Simon, 2015; Barragan-Fonseca et al., 2017). The reason for the rapid decrease in CP from days 0 to 11 BSFL may be due to the normal growth and development of the body that occurs at that time. The body size of BSFL increases from only 1 mm at hatching to ~27 mm in length and 6 mm in width before the prepupal stage (Park, 2016).

Crude fat (**CF**) content of BSFL is also affected by the protein and carbohydrate content of the diet fed. According to Barragan-Fonseca et al. (2019), BSFL CF content increased when BSFL were fed a diet containing high CP (24%) and carbohydrate (55%). BSFL CF content of different life stages was evaluated by Liu et al. (2017), and CF content tended to fluctuate. They reported that CF firstly decreased after hatching (15.8%) to the neonate larvae (11.8%) and gradually increased in the larval phase (28%), and then reduced in pupal stages. In the current study, the AHF content of BSFL increased from days 0 to 14 and then decreased from days 18 to 29. The reason for the increase of fat content from days 0 to 14 may be due to their energy needs for metamorphosis during the pupal stages (Mirth and Riddiford, 2007; Arrese and Soulages, 2010). Moreover, because adult BSFL do not feed during the time of mating and oviposition, the amount of nutrients accumulated in the larval stage is essential for adult life (Arrese and Soulages, 2010; Liu et al., 2017). In this study, ash content linearly increased from days 0 to 29, and this result was similar to the value previously reported by Liu et al. (2017). The possible reason for a higher level of ash in pupal than in larval stage is due to the formation of the cuticle layer in their body (Veldkamp et al., 2012; Liu et al., 2017).

BSFL contain about 41% to 44% CP, but less than house flies (45% to 55% CP) and mealworms (49% to 57% CP) (Klasing et al., 2000; St-Hilaire et al., 2007; Makkar et al., 2014). St-Hilaire et al. (2007) compared AA concentrations of the BSFL prepupal and pupal stages of houseflies. Most of the indispensable and dispensable AA concentrations of BSFL were lower than that of house flies, but not significantly. Of course, the methodology used to rear the insects and analyze the composition in the lab will impact nutrient concentrations measured. Compared with the AA concentrations of larval and prepupal stages of BSFL fed household organic waste evaluated by Kawasaki et al. (2019), some of indispensable AA of prepupal stage (arginine, histidine, leucine, methionine, threonine, and tryptophan) were higher than day 23 BSFL in this study. Also, some of the indispensable AA in the larval stage (arginine, histidine, isoleucine, and leucine) were higher than day 18 BSFL in this study. However, the prepupal stages of BSFL fed swine manure had lower indispensable AA such as leucine, methionine, and tryptophan than day 23 BSFL (Newton et al., 2005). Additionally, prepupa reared on dairy cow manure showed lower indispensable AA (isoleucine, methionine, phenylalanine, and threonine) than day 23 BSFL (Sealey et al., 2011). These results demonstrate the importance of diet and environment on BSFL AA and protein composition.

When evaluating protein quality of feed ingredients, AA and macronutrient composition and digestibility are necessary. Therefore, ileal-cannulated animals and the cecectomized rooster assay have been used to determine the quality of protein ingredients (Johnson et al., 1998; Faber et al., 2010; Kerr et al., 2014; Oba et al., 2019). Although the precision-fed cecectomized rooster assay includes a surgical procedure and does not allow the measure of AA metabolism, this assay is a good model to measure the nutrient and AA digestibility of pet food ingredients because ileal-cannulated dog and cat models have issues related to expense, animal welfare, and length of time (Engster et al., 1985; Kerr et al., 2014; Deng et al., 2016). Additionally, the results from the cecectomized rooster assay and ileal-cannulated dogs have been shown to be highly correlated. According to Johnson et al. (1998), they used six ileal-cannulated dogs (6 × 6 Latin square design) and 24 cecectomized roosters (completely randomized design) to test six animal by-product foods, with results showing a high correlation between roosters and dogs (r = 0.87 to 0.92). Therefore, the cecectomized rooster assay is an appropriate model to evaluate the protein quality of different ages of BSFL ingredients (Johnson et al., 1998; Kerr et al., 2014; Deng et al., 2016; Oba et al., 2019).

For all AA evaluated in this study, days 14, 18, and 29 BSFL had the higher AA digestibilities than other stages of BSFL. Of the indispensable AA, days 14, 18, and 23 BSFL had a digestibility of over 90% except for histidine, threonine, and valine. Besides days 0, 11, and 29, BSFL had an indispensable AA digestibility of over 85% except for histidine and valine. Although days 14, 18, and 23 BSFL had higher indispensable AA digestibilities than other stages of BSFL for most AA, all BSFL ingredients could be easily absorbed and utilized by roosters.

Little information is available about the AA digestibility of different ages of BSFL. When compared with the data conducted by De Marco et al. (2015), all AA digestibilities of the BSFL meal had lower values (<85%) because the larvae were dried for 20 h at low temperature (60 °C) and ground to meal and also, they were reared on a different diet (cereal by-product) compared with the current study. The defatted BSFL reported by Mwaniki and Kiarie (2018) had similar AA digestibility to the day 11 BSFL in the present study but lower than days 14, 18, and 29 BSFL except for valine and glycine. BSFL diet, processing methods, chitin contents in the prepupal and pupal stage, experimental design and environmental factors (temperature and humidity) may affect AA digestibility of BSFL.

DIAAS-like values have recently been used to evaluate the protein quality of feed ingredients for dogs and cats (Oba et al., 2019). Based on the DIAAS-like values in this study, if a diet was formulated with only BSFL protein for growing puppies by using AAFCO nutrient requirements, day 14 BSFL may not provide sufficient arginine (DIAAS-like value = 98.68), methionine (DIAAS-like value = 89.48), phenylalanine (DIAAS-like value = 95.12), and threonine (DIAAS-like value = 75.26), while days 18 and 23 BSFL need more threonine (DIAAS-like value = 80.34; 77.7) to meet nutrient requirement. If NRC recommendations are used as a reference, only methionine (DIAAS-like value = 94.9) and threonine (DIAAS-like value=96.63) were the limiting AA in day 14 BSFL. For the growth and reproduction of cats and kittens, only methionine (DIAAS-like value = 64.45 to 96.7) was not sufficient if a diet was formulated based on AAFCO and NRC recommendations. As a similar pattern was observed in adult dogs using AAFCO recommendations, all BSFL ingredients fulfill the AA requirements except for methionine (DIAAS-like value = 72.65 to 92.84). However, based on the NRC recommendations, day 14 BSFL had sufficient arginine (DIAAS-like value = 125.31), histidine (DIAAS-like value = 140.78), isoleucine (DIAAS-like value = 102.88), lysine (DIAAS-like value = 176.10), and valine (DIAAS-like value = 114.70). Days 18 and 23 BSFL also showed a similar pattern with day 14 BSFL. For adult cats, all BSFL ingredients had DIAAS-like values over 100%.

In conclusion, this study provided the macronutrient and AA digestibilities data of different ages of BSFL intended for use in dog and cat foods. DM and OM digestibilities had no difference, but AHF digestibility tended to vary and AA digestibilities were different among substrates. For evaluating protein quality of different ages of BSFL, our data showed that AA digestibilities were highest in days 14, 18, and 23 BSFL and threonine, methionine, and tryptophan were often the first-limiting AA of BSFL based on DIAAS-like values for dogs and cats. However, all ages of BSFL ingredients had high-quality protein, AA concentrations, and digestibilities. Therefore, BSFL appears to be a high-quality protein source for pet food. These data provide more information into the potential for BSFL to be applied to pet food and other animal feeds. Differences in rearing conditions, diet, and processing characteristics need to be taken into account when utilizing a commercial source of BSFL, but it is clear that producers and their customers may be able to modify the nutrient content and subsequent digestibility of insect-derived ingredients for a particular application. Further research such as product safety, pet owner perception, processing effects (extrusion and retort), and nutrient digestibility of a complete and balanced diet including BSFL as a significant protein source should be done to determine how much BSFL may be used in pet food formulations.

Conflict of interest statement. L.K. is employed by EnviroFligh. All other authors have no conflicts of interest.

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