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Palatability and apparent total tract macronutrient digestibility of retorted black soldier fly larvae-containing diets and their effects on the fecal characteristics of cats consuming them

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Abstract

There is a growing interest in using black soldier fly larvae (**BSFL**) due to its supposed sustainability and nutritional qualities. Because little research has been conducted to evaluate the use of BSFL in cats, our objective was to determine the palatability and apparent total tract macronutrient digestibility (**ATTD**) of BSFL-containing canned diets and the fecal characteristics of healthy adult cats consuming them. First, three palatability tests were conducted to compare the following diets: 1) diet with poultry byproduct meal (**PBPM**) and chicken serving as the primary protein sources (control) vs. diet with BSFL meal replacing PBPM (BSFL meal); 2) control vs. diet with whole BSFL replacing some PBPM and poultry fat (BSFL whole); and 3) control vs. diet with BSFL oil replacing poultry fat (BSFL oil). All diets were formulated to meet Association of American Feed Control Officials nutrient profiles for adult cats and were produced using a still retort. A paired *t*-test was conducted to analyze data from each palatability test, with a higher ($P < 0.05$) consumption ratio being observed for BSFL meal (1.93:1), BSFL whole (2.03:1), and BSFL oil (1.57:1). Second, 32 adult cats (20 females and 12 males; BW: 4.19 ± 0.55 kg; age: 3.3 ± 0.38 yr) were used in a completely randomized design study composed of a 21-d baseline period and a 70-d experimental period. Cats consumed the control diet during the baseline and were then allotted to one of four experimental diets ($n = 8$ per group): 1) control, 2) BSFL meal, 3) whole BSFL, and 4) BSFL oil. Fecal samples were collected after baseline and experimental periods for ATTD and fecal characteristic analysis. Fecal output was higher ($P < 0.05$) and fecal dry matter percentage was lower ($P < 0.05$) in cats fed BSFL meal than those fed BSFL oil. Organic matter, crude protein (**CP**), and energy ATTD were lower ($P < 0.05$) in cats fed BSFL meal than those fed BSFL oil or control. CP and energy ATTD were lower ($P < 0.05$) in cats fed BSFL whole than those fed BSFL oil. A few serum metabolites were affected by diet ($P < 0.05$) but remained within reference ranges. Hematology was not affected by diet ($P > 0.05$). Overall, our results suggest that BSFL-containing diets are palatable and do not negatively affect fecal characteristics or serum chemistry but may have slightly lower nutrient digestibilities in adult cats.

Lay Summary

The pet food industry is interested in black soldier fly larvae (**BSFL**) because of its supposed sustainability and nutritional qualities. BSFL has not been well tested in cats, so our objective was to determine the palatability and apparent total tract macronutrient digestibility (**ATTD**) of BSFL-containing canned diets and how they affected the fecal characteristics of adult cats. Palatability tests were conducted to compare diets containing BSFL meal, whole BSFL, or BSFL oil against a poultry-based control. Higher consumption ratios were observed for all BSFL-containing diets. A second study determined the digestibility of BSFL-containing diets and how they affected fecal characteristics. Cats were allotted to a control diet or diets containing BSFL meal, whole BSFL, or BSFL oil. Fecal output was higher and fecal dry matter percentage was lower in cats fed BSFL meal than those fed BSFL oil. Organic matter, crude protein (**CP**), and energy ATTD were lower in cats fed BSFL meal than those fed BSFL oil or control. CP and energy ATTD were lower in cats fed whole BSFL than those fed BSFL oil. Overall, BSFL-containing diets were shown to be palatable and did not negatively affect fecal characteristics, but reduced nutrient digestibilities in adult cats.

Key words: feline nutrition, insect meal, pet food

Abbreviations: AAFCO, Association of American Feed Control Officials; AHF, acid-hydrolyzed fat; ATTD, apparent total tract digestibility; CP, crude protein; DM, dry matter; DMB, dry matter basis; OM, organic matter; PBPM, poultry byproduct meal; TDF, total dietary fiber

Introduction

Interest in black soldier fly larvae (**BSFL**; *Hermetia illucens*) as an alternative protein and fat source for pet food has increased recently because of its high protein and fat content and its supposed low environmental footprint (crude protein [CP]: 38.5% to 47.9%; crude fat: 14.6% to 39.2%; Zheng et al., 2012; Bosch et al., 2014; Nguyen et al., 2015; Bosch and Swanson, 2021). Previous studies using a cecectomized

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rooster model have demonstrated that BSFL has high amino acid (**AA**) digestibility and protein quality (Do et al., 2020, 2021). In addition to serving as a high-quality protein source, the biofunctionalities of BSFL-derived proteins have been evaluated recently. These proteins have been shown to have anti-inflammatory, antioxidant, immunomodulatory, and antibacterial properties (Firmansyah and Abduh, 2019; Mouithys-Mickalad et al., 2020; Zhu et al., 2020). For example, several peptide sequences present in BSFL hydrolysates that have alanine, lysine, isoleucine, phenylalanine, leucine, and valine residues at the C- and N-termini have been shown to enhance free radical scavenging ability (Zhu et al., 2020). BSFL oil is also a unique fat source because it is high in medium-chain fatty acids such as lauric acid (C12:0). These fatty acids have antibacterial properties and are an important component of sebaceous lipids (sebum) that provide protection to the skin from pathogens and water loss (Fischer, 2020). Moreover, chitin that is present in the cuticle of BSFL (structurally identical to cellulose), has been suggested to have prebiotic potential that may benefit gut health (Roseland et al., 1985; Tomberlin et al., 2002; Finke, 2013). These potential impacts on health require further study in general and in the target species.

A few studies have evaluated the use of BSFL in pet foods, with the main focus being on dogs thus far. With most novel ingredients, palatability, gastrointestinal tolerance, and apparent total tract digestibility (**ATTD**) are the initial outcomes of interest. Compared with dog foods based on traditional protein sources, foods containing various insects such as BSFL, mealworms (*Tenebrio molitor*), crickets (*Gryllodes sigillatus*), and cockroaches (*Shelfordella lateralis*) have been shown to have similar smell and consumption preferences by dogs. For example, when beagle dogs had free access to commercial dry dog foods with aromas from four insect species, results showed no differences ($P > 0.05$) in the frequency of first choice (Kierończyk et al., 2018). Beynen (2018) reported food consumption results, with dogs slightly preferring a BSFL-containing diet over a yellow mealworm-containing diet (intake ratio = 60:40), while the reverse was noted in cats (intake ratio = 40:60).

Four published studies have tested the digestibility of BSFL-containing diets in dogs. In one study, adult female beagles (n = 9) fed extruded diets containing 0%, 1%, and 2% of BSFL meal for 42 d had linear increases in dry matter (**DM**) and CP ATTD, but fat ATTD was not changed (Lei et al., 2019). In another study, DM ATTD was slightly greater ($P < 0.05$; 83% vs. 82%) while CP digestibility was slightly lower ($P < 0.05$; 77% vs. 79%) in beagle dogs fed a BSFL meal-containing extruded diet (20% in diet, DM basis [**DMB**]) than those fed a control diet based on lamb meal (Kröger et al., 2020). When adult dogs were fed an extruded diet containing 28% BSFL meal (DMB), they had higher ($P < 0.05$) CP (82.3%) and fat (94.5%) ATTD compared with those fed a poultry-based diet (33% in diet, DM; El-Wahab et al., 2021). Finally, Freel et al. (2021) tested BSFL meal-based extruded diets (5%, 10%, and 20% in diet, as-is) and BSFL oil-based diets (2.5% and 5.0% in diet, as-is) in adult dogs, reporting that there were no differences in the ATTD of DM, CP, fat, and energy among treatments.

Other than two studies testing extruded diets (Paßlack and Zentek, 2018; Pezzali and Shoveller, 2021), limited research has been conducted to evaluate BSFL-containing in cats and with no studies testing canned diets. For this reason, the objective of this study was to determine the palatability and ATTD of BSFL-containing canned diets and the fecal characteristics of healthy adult cats consuming them. We hypothesized that when compared with a poultry-based control diet, BSFL-containing canned diets would not negatively affect palatability, ATTD, or fecal characteristics of healthy adult cats.

Materials and Methods

All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee prior to experimentation (IACUC #19268).

Experimental diets

Experimental diets included: 1) diet based on fresh/frozen poultry, poultry byproduct meal (**PBPM**), corn gluten meal, white rice, chicken liver, and poultry fat (control); 2) diet with BSFL meal primarily replacing PBPM and with slight adjustments to corn gluten meal and white rice (BSFL meal; providing ~42% of CP and ~36.5% of fat); 3) diet with whole BSFL replacing some PBPM and poultry fat and with slight adjustments to corn gluten meal and white rice (BSFL whole; providing ~17% of CP and ~38% of fat); and 4) diet with BSFL oil replacing poultry fat and with slight adjustments to PBPM, deboned chicken, and corn gluten meal (BSFL oil; providing ~22% of fat). All experimental diets were formulated to meet all Association of American Feed Control Officials (AAFCO, 2019) nutrient recommendations for adult cats, included fresh/frozen poultry, PBPM, white rice, chicken liver, and poultry fat, and were formulated to contain approximately 40% CP and 20% crude fat on DMB. Diets were manufactured at the University of Illinois Integrated Bioprocessing Research Laboratory. Briefly, ingredients for each diet were first weighed and mixed by hand before being homogenized with opposite turning agitators in a jacked steam kettle heated to 65 °C for 15 to 20 min. The cooked mixture was then filled into cans (500 ± 5 g) and steam-flushed prior to sealing. Sealed cans were then retorted in an Allpax Gentle Motion Retort (30802 series, Allpax, Covington, LA). The vessel was maintained at constant pressure (18 psi) and temperature (121 °C) until desired kill temperature was reached. All diets were prepared using the same conditions.

Palatability studies

Three 2-d palatability tests were conducted at Kennelwood Inc. (Champaign, IL). In each test, 20 adult cats (BW: 4.56 ± 1.18 kg) were used. Two stainless steel bowls, each containing approximately 150 g of canned diet, were offered once daily for 2 d. Bowl placement was reversed daily to prevent left–right bias and both bowls were presented for 30 min. If one diet was completely consumed prior to the end of the 30 min, both bowls were removed. Food consumption and first choice preference were recorded for each cat. The following experiments were performed: 1) control vs. BSFL meal; 2) control vs. BSFL whole; and 3) control vs. BSFL oil. A paired t-test was conducted to analyze data from the palatability tests, with statistical significance set as $P < 0.05$.

Digestibility study

Animals and housing

Healthy adult cats (20 females and 12 males; BW: 4.19 ± 0.55 kg; age: 3.3 ± 0.38 yr) were used in a completely randomized design. All cats were housed in cages (approximately 1.02 m deep; 0.76 m wide; 0.71 m high) during feeding times at the University of Illinois at a temperature (21 °C) and light-controlled (14 h light:10 h dark) room. At other times, cats were group-housed outside of their cages. Cats were allowed access to various toys and scratching poles for environmental enrichment and were socialized with humans at least 2 times per wk. On the basis of the estimated maintenance energy requirement for adult cats and information from previous feeding records, an amount of food to maintain BW was offered and intake was measured twice daily (8:00 a.m. to 9:00 a.m. and 3:00 p.m. to 4:00 p.m.). Cats had free access to fresh water at all times.

Experimental periods

This study was composed of a 21-d baseline period and a 70-d experimental period. During the baseline, all cats consumed the control diet (Table 1). After baseline measurements, cats were randomly assigned to one of the four experimental canned diets (n = 8 per group): 1) control, 2) BSFL meal, 3) BSFL whole, and 4) BSFL oil. Cats were fed to maintain BW throughout the study. The food offered and refusals were measured daily to calculate intake.

Fecal and blood sample collection and analyses

During the fecal collection phase, total fecal samples were collected for 5 d. At these times, total feces were weighed and frozen at −20 °C until analyses were completed. Fresh feces (within 15 min of defecation) were collected for measurement of pH and moisture content. Fecal pH was measured immediately using an AP10 pH meter (Denver Instrument, Bohemia, NY) equipped with a Beckman Electrode (Beckman Instruments Inc., Fullerton, CA). An aliquot was then collected for DM determination. All fecal samples during the collection phase were scored according to the following scale: 1 = hard, dry pellets, small hard mass; 2 = hard, formed, dry stool, remains firm and soft; 3 = soft, formed, and moist stool, retains shape; 4 = soft, unformed stool, assumes the shape of the container; and 5 = watery, the liquid that can be poured.

On the last day of each collection period, approximately 4 mL of blood was collected via jugular or cephalic puncture. Cats were fasted for 12 h prior to blood collection. Cats were sedated by intramuscular injection of dexmedetomidine (0.5 mg/mL IM) for sedation. After blood was collected, an injection of the reversal agent for dexmedetomidine, atipamezole (0.5 mg/mL intramuscular), was given. Samples were immediately transferred to appropriate vacutainer tubes. The blood tubes for serum isolation were centrifuged at 1,300 × g at 4 °C for 10 min (Beckman CS-6R centrifuge; Beckman Coulter Inc., Brea, CA). Serum was then transported to the University of Illinois Veterinary Medicine Diagnostics Laboratory for serum chemistry analysis. K_2EDTA tubes were cooled (but not frozen) and then transported to the University of Illinois Veterinary Medicine Diagnostics Laboratory for hematology. Cats received their regular daily meal after blood collection.

Chemical analysis and ATTD calculations

Diet subsamples were collected, lyophilized, and ground through a 2-mm screen using a Wiley Mill (model 4, Thomas Scientific, Swedesboro, NJ) with dry ice to allow for proper grinding before analysis. Total fecal samples were composited and dried at 57 °C for a week. Fecal samples were then ground through a 2-mm screen using a Wiley Mill (model 4). DM and ash concentrations were analyzed according to the Association of Official Analytical Chemists, with organic matter (**OM**) being calculated (AOAC, 2006; DM: method 934.01; OM: method 942.05). Fat concentrations were measured by acid hydrolysis according to the AACC (1983) followed by diethyl ether extraction (Budde, 1952). CP concentration was

Table 1. Ingredient composition of the experimental diets fed to adult cats (%, as-is)

Ingredient	Control	BSFL meal	BSFL whole	BSFL oil
Water	63.33	63.65	63.36	62.63
Black soldier fly larvae (BSFL) meal	-	10.00	-	-
BSFL (whole)	-	-	5.00	-
BSFL oil	-	-	-	1.50
Poultry byproduct meal	9.34	-	4.00	8.42
Chicken, deboned without skin	10.50	10.50	10.50	11.50
Corn gluten meal	4.00	5.00	5.20	4.62
Chicken liver	4.00	4.00	4.00	4.00
Poultry fat	1.50	1.50	1.00	-
White rice	5.00	3.03	4.62	5.00
Guar gum	0.60	0.60	0.60	0.60
Rice flour	1.50	1.50	1.50	1.50
Mineral premix[1]	0.07	0.07	0.07	0.07
Vitamin premix[2]	0.06	0.06	0.06	0.06
L-taurine	0.05	0.05	0.05	0.05
Salt	0.05	0.05	0.05	0.05

[1]Provided per kg diet: Cu (as $CuCO_3$), 7.0 mg; K (as KIO_3), 0.7 mg; Fe (as $C_6H_5FeO_7$), 52.5 mg; Mn (as $MnCO_3$), 7.0 mg; Na (as Na_2SeO_3), 154.0 mg; Zn (as $ZnCO_3$), 70.0 mg; Co (as $CoSO_4$), 1.5 mg.
[2]Provided per kg diet: vitamin A, 6,000 IU; vitamin D3, 900 IU; vitamin E, 48 IU; vitamin K3, 0.72 mg; biotin, 0.04 mg; vitamin B12, 38.33 µg; folic acid, 0.36 mg; nicotinic acid, 41.40 mg; vitamin B5, 16.80 mg; pyridoxine, 10.20 mg; riboflavin, 10.20 mg; thiamin, 10.20 mg.

calculated from Leco total nitrogen values (TruMac N, Leco Corporation, St. Joseph, MI; AOAC, 2006). Gross energy (**GE**) was measured using an oxygen bomb calorimeter (model 6200, Parr Instruments, Moline, IL). Total dietary fiber (**TDF**) concentration of the diet samples was determined according to Prosky et al. (1985). Chitin content of BSFL meal and whole BSFL was measured according to Muzzarelli (1977). ATTD of nutrients and energy was calculated using the following equation: Digestibility (%) = (Nutrient intake [g/d] − Fecal output [g/d])/Nutrient intake (g/d) × 100%.

Statistical analyses

All data were analyzed using the Mixed Models procedure of SAS (version 9.4; SAS Institute, Cary, NC) with treatment as a fixed effect and cat as a random effect. Differences among treatments were determined using a Fisher-protected least significant difference with a Tukey adjustment to control for experiment-wise error. Data are reported as means ± pooled SEM with statistical significance set as $P < 0.05$.

Results

Chemical composition

The chemical composition of the diets tested is presented in Table 2. The DM content among experimental diets was similar (25.5% to 28.8%, DMB). On DMB, OM content was highest in the BSFL meal diet (95.9%) and lowest in the control diet (92.4%). The acid-hydrolyzed fat (**AHF**) and GE were lowest for the control diet (15.2%, 4.75 kcal/g DMB) and similarities were observed in the BSFL meal (17.2%, 4.93 kcal/g DMB), BSFL whole (18.2%, 4.93 kcal/g DMB), and BSFL oil (17.1%, 4.93 kcal/g DMB) diets. The control and BSFL oil diets had a higher CP content (42.2% and 42.7% DMB) than the BSFL whole diet (39.4% DMB), followed by the BSFL meal diet (33.4% DMB). The TDF content was highest for the BSFL whole diet (7.6% DMB), followed by the BSFL meal (7.3% DMB) and control (5.0% DMB) diets, and lowest in the BSFL oil diet (4.7% DMB).

Palatability testing

In Experiment 1, the BSFL meal diet (92.6 ± 9.1 g/d) was preferred ($P = 0.0086$) by a 1.93:1 intake ratio over the control diet (48.0 ± 9.1 g/d). Individual food intakes are presented in Supplementary Figure S1. In addition to having higher consumption, the BSFL meal diet was first approached 28 out of 40 occasions and first consumed 30 out of 40 occasions over the 2 d. In Experiment 2, the BSFL whole diet (100.6 ± 9.1 g/d) was preferred ($P = 0.0023$) by a 2.03:1 intake ratio over the control diet (49.5 ± 8.8 g/d). Individual food intakes are presented in Supplementary Figure S2. Over the 2 d, the BSFL whole diet was first approached 25 out of 40 occasions and first consumed 27 out of 40 occasions. In Experiment 3, the BSFL oil diet (85.5 ± 8.6 g/d) was preferred ($P = 0.0297$) by a 1.57:1 intake ratio over the control diet (54.5 ± 8.3 g/d). Individual food intakes are presented in Supplementary Figure S3. Over the 2 d, the BSFL oil diet was first approached on 24 out of 40 occasions and first consumed 24 out of 40 occasions.

Food intake, fecal output, fecal characteristics, and ATTD

Food intake, fecal characteristics, and ATTD data are presented in Table 3. There were no differences in food or caloric intake ($P > 0.05$) among diets. However, cats fed the BSFL meal diet had a higher ($P < 0.05$) fecal output (as-is and DMB) than cats fed the BSFL oil diet. Similarly, cats fed the BSFL meal diet had a higher ($P < 0.05$) fecal caloric output (kcal/d) than cats fed the control or BSFL oil diets. Fecal pH and scores were not different among diets. However, cats fed the BSFL oil diet had a higher ($P < 0.05$) fecal DM than cats fed the BSFL meal diet. The ATTD of DM was greater ($P < 0.05$) in cats fed the BSFL oil diet than those fed the BSFL meal diet. The ATTD of OM was greater ($P < 0.05$) in cats fed the control or BSFL oil diets than those fed the BSFL meal or BSFL whole diets. For CP, ATTD was greater ($P < 0.05$) in cats fed the BSFL oil diet than those consuming the BSFL meal or BSFL whole diets. ATTD of CP was also greater ($P < 0.05$) in cats fed the control or BSFL whole diets than those fed the BSFL meal diet. The ATTD of energy was greater ($P < 0.05$) in cats fed the BSFL oil diet than those consuming the BSFL meal or BSFL whole diets. The ATTD of energy was also greater ($P < 0.05$) in cats fed the control diet than those consuming the BSFL meal diet. There were no differences in the ATTD of AHF among groups ($P > 0.05$).

Table 2. Analyzed chemical composition of experimental diets fed to adult cats

Item	Treatment			
	Control	BSFL meal	BSFL whole	BSFL oil
Dry matter (DM), %	28.4	28.2	25.5	28.8
		DM basis		
Organic matter, %	92.4	95.9	94.4	93.2
Ash, %	7.6	4.1	5.6	6.8
Acid-hydrolyzed fat, %	15.2	17.2	18.2	17.1
Crude protein, %	42.2	33.4	39.4	42.7
Total dietary fiber, %	5.0	7.3	7.6	4.7
Choline, mg/kg	2,020	2,060	2,040	1,930
Taurine, %	0.2	0.2	0.3	0.3
Chitin[1], %	0.0	0.9	1.0	0.0
Gross energy, kcal/g	4.75	4.93	4.93	4.93

[1]Chitin content of diets estimated based on chitin content of BSFL meal (2.41%) and BSFL whole (4.91%).

Table 3. Food intake, fecal characteristics, and apparent total tract macronutrient digestibility of adult cats fed black soldier fly larvae (BSFL)-containing canned diets

Item	Treatment				SEM[1]	*P*-value
	Control	BSFL meal	BSFL whole	BSFL oil		
Food intake						
g food/d (as-is)	216.00	208.05	205.01	202.62	4.131	0.7042
g food/d (DM basis)	61.28	58.63	52.34	58.78	1.271	0.0778
kcal/d	290.86	289.32	258.00	287.32	6.038	0.1679
Fecal output						
g feces/d (as-is)	30.55[ab]	39.94[a]	30.40[ab]	23.82[b]	1.593	0.0015
g feces/d (DM basis)	8.88[ab]	10.98[a]	8.86[ab]	7.83[b]	0.400	0.0327
kcal/d	36.06[b]	49.58[a]	41.25[ab]	32.88[b]	1.890	0.0053
Fecal characteristics						
Fecal pH	6.56	6.63	6.64	6.68	0.024	0.3619
Fecal score[2]	2.88	2.50	2.75	2.31	0.083	0.0674
Fecal DM, %	28.92[ab]	27.91[b]	29.65[ab]	33.00[a]	0.701	0.0499
Digestibility, %						
Dry matter	85.47[ab]	81.19[b]	83.16[ab]	86.51[a]	0.655	0.0118
Organic matter, %	88.63[a]	83.34[b]	84.80[b]	89.15[a]	0.651	0.0005
Acid-hydrolyzed fat, %	88.94	87.64	87.75	90.61	0.552	0.1976
Crude protein, %	86.71[ab]	77.73[c]	82.87[b]	87.64[a]	0.888	<0.0001
Energy, %	87.57[ab]	82.79[c]	84.10[bc]	88.52[a]	0.645	0.0007

[1]Pooled standard error of the mean.
[2]Fecal scores: 1 = hard, dry pellets, small hard mass; 2 = hard formed, remains firm and soft; 3 = soft, formed and moist stool, retains shape; 4 = soft, unformed stool, assumes the shape of the container; 5 = watery, liquid that can be poured.
[a,b,c]Within a row, means lacking a common superscript differ ($P < 0.05$).

Serum chemistry profiles and hematology

Serum chemistry profiles and hematology of cats fed all diets were within the reference ranges for adult cats, with the exception of a slightly higher albumin to globulin ratio (reference range: 0.6 to 1.1; baseline: 1.15, BSFL oil: 1.13; 7 cats outside range), slightly lower blood Ca (reference range: 8.8 to 10.2 mg/dL; BSFL whole: 8.64 mg/dL, control: 8.70 mg/dL; 16 cats outside range), slightly higher Na to K ratio (reference range: 28 to 36; baseline: 38.28, control: 40.25; 8 cats outside range), slightly higher cholesterol (reference range: 66 to 160 mg/dL; BSFL meal: 189.37 mg/dL, BSFL whole: 173.13 mg/dL; 16 cats outside range), and slightly higher eosinophils (reference range: 0 to 0.8 × 10^3 per µL; baseline: 0.88 × 10^3 per µL, control: 0.91 × 10^3 per µL, BSFL meal: 0.86 × 10^3 per µL; 13 cats outside range; Tables 4 and 5). Cats fed the control diet had greater ($P < 0.05$) serum creatinine concentrations than those fed the BSFL meal or BSFL whole diets. Cats fed the control or BSFL meal diets had higher ($P < 0.05$) blood urea nitrogen concentrations than those fed the BSFL whole diet. Cats fed the BSFL meal diet had higher ($P < 0.05$) serum bicarbonate concentrations than those fed the control diet. All other blood metabolites and cells were not affected among groups ($P > 0.05$).

Discussion

The use of BSFL-based ingredients in animal feed is promising due to their supposed low environmental footprint, high protein content, and high lipid content (Zheng et al., 2012; Bosch et al., 2014; Makkar et al., 2014; Nguyen et al., 2015; Wang and Shelomi, 2017; Bosch and Swanson, 2021). In previous studies, our lab used a cecectomized rooster model to evaluate the AA digestibility and protein quality of BSFL-based ingredients (Do et al., 2020, 2021). Digestible indispensable AA score-like values were calculated according to Mathai et al. (2017) using AAFCO nutrient profiles and NRC recommended allowances for dogs and cats and were shown to be of high quality, with limiting AA being methionine and cysteine for most ingredients (Do et al., 2020, 2021). In regard to fat, BSFL oil derived from the defatting process is high in saturated fatty acids (52.5% to 68.7% of total fatty acids), followed by monounsaturated fatty acids (12.2% to 22.2% of total fatty acids) and polyunsaturated fatty acids (7.8% to 24.2% of total fatty acids; Barroso et al., 2017; Liland et al., 2017; Kierończyk et al., 2020).

A few studies have recently evaluated BSFL-containing diets in dogs. El-Wahab et al. (2021) reported that dogs fed a BSFL meal-based diet (30% of BSFL meal, DMB) had higher ($P < 0.05$; 114 g/d as-is) fecal output than dogs fed a PBPM-based diet (30% of PBPM, DMB; 98.4g/d as-is). Even though ATTD of CP (80.5% vs. 82.3%) and crude fat (CF; 91.6% vs. 94.5%) were higher ($P < 0.05$) in dogs fed a BSFL meal-based diet than those fed a PBPM-based diet, no differences ($P > 0.05$) were observed in ATTD of OM and nitrogen free extract (**NFE**) in that study. Kröger et al. (2020) reported that dogs eating a BSFL-meal diet (20% of diet, DMB) had a lower ($P < 0.05$) daily fecal output (109 g/d, as-is) than those fed the control diet (lamb meal as the main protein source; 137 g/d, as-is). In that study, dogs fed a BSFL-meal diet had a lower ($P > 0.05$) ATTD of CP (79.2% vs. 77.3%) than dogs fed the control diet. ATTD of DM and NFE showed an opposite result, with dogs eating a BSFL-meal diet having higher

Table 4. Serum chemistry profiles for adult cats fed black soldier fly larvae (BSFL)-containing canned diets

Item	Reference range[1]	Treatment				SEM[2]	*P*-value
		Control	BSFL meal	BSFL whole	BSFL oil		
Creatinine, mg/dL	0.4–1.6	1.43[a]	1.15[b]	1.13[b]	1.30[ab]	0.035	0.0047
Blood urea nitrogen, mg/dL	18–38	28.13[a]	28.88[a]	23.00[b]	26.63[ab]	0.668	0.0044
Total protein, g/dL	5.8–8.0	6.18	6.08	5.86	6.24	0.080	0.3838
Albumin, g/dL	2.8–4.1	3.20	3.04	3.06	3.29	0.047	0.1966
Globulin, g/dL	2.6–5.1	2.98	3.04	2.80	2.95	0.060	0.5755
Albumin:globulin ratio	0.6–1.1	1.09	1.01	1.10	1.13	0.027	0.5271
Ca, mg/dL	8.8–10.2	8.70	8.84	8.64	8.79	0.058	0.6440
P, mg/dL	3.2–5.3	3.96	3.88	3.68	4.23	0.102	0.3032
Na, mmol/L	145–157	147.13	148.25	147.37	147.25	0.258	0.4179
K, mmol/L	3.6–5.3	3.77	4.49	4.23	4.23	0.106	0.1167
Na:K ratio	28–36	40.25	33.88	35.38	34.75	1.186	0.2296
Cl, mmol/L	109–126	118.00	117.38	117.38	116.25	0.327	0.3017
Alkaline phosphatase, U/L	10–85	20.50	23.00	22.38	24.75	1.327	0.7977
Alanine transaminase, U/L	8–65	57.75	46.13	38.25	64.25	4.830	0.2300
Gamma glutamyl transferase, U/L	0–3	0.00	0.00	0.13	0.13	0.043	0.5796
Total bilirubin, mg/dL	0.0–0.3	0.14	0.15	0.13	0.15	0.009	0.7296
Creatine phosphokinase, U/L	10–250	140.25	125.00	168.13	153.50	12.562	0.6773
Cholesterol, mg/dL	66–160	145.62	189.37	173.13	158.00	6.830	0.1187
Triglycerides, mg/dL	21–166	33.00	28.75	50.13	34.50	4.549	0.3813
Bicarbonate, mmol/L	12–21	17.75[b]	20.38[a]	19.00[ab]	19.00[ab]	0.316	0.0255
Anion gap	10–27	15.00	15.00	15.25	16.00	0.275	0.5474

[1]University of Illinois Veterinary Diagnostic Laboratory Reference Ranges.
[2]Pooled standard error of the mean.
[a,b]Within a row, means lacking a common superscript differ ($P < 0.05$).

Table 5. Hematology of adult cats fed black soldier fly larvae (BSFL)-containing canned diets

Item	Reference range[1]	Treatment				SEM[2]	*P*-value
		Control	BSFL meal	BSFL whole	BSFL oil		
Red blood cells, 10^6 per uL	5.00–10.00	8.53	8.62	7.86	8.26	0.130	0.1567
Reticulocyte count, %	-	0.11	0.12	0.08	0.12	0.012	0.4768
Hemoglobin, g/dL	8.0–15.0	12.34	12.40	11.64	11.8	0.206	0.4803
Hematocrit, %	30.0–55.0	35.28	36.28	33.34	33.74	0.576	0.2442
Mean cell volume, fi	37.0–55.0	41.41	42.43	42.36	40.9	0.372	0.5084
Mean corpuscular hemoglobin, pg	13.0–18.0	14.50	14.41	14.79	14.3	0.154	0.7303
Mean corpuscular hemoglobin, g/dL	29.0–38.0	34.98	34.23	34.90	34.93	0.211	0.5647
White blood cells, 10^3 per µL	5.50–19.50	12.88	12.31	10.59	11.95	2.876	0.7412
Lymphocytes, 10^3 per µL	1.70–7.00	3.68	3.63	3.22	4.71	0.317	0.4016
Lymphocytes, %	-	28.98	29.26	32.46	39.63	1.905	0.1651
Monocytes, 10^3 per µL	0.00–0.90	0.42	0.30	0.41	0.35	0.026	0.3671
Monocytes, %	-	3.36	2.89	4.30	3.11	0.300	0.3737
Eosinophils, 10^3 per µL	0.00–0.80	0.91	0.86	0.70	0.69	0.058	0.4544
Eosinophils, %	-	7.80	7.48	7.19	6.03	0.660	0.8103
Basophils, 10^3 per µL	0.0–2.0	0.07	0.01	0.03	0.03	0.008	0.0673
Basophils, %	-	0.73	0.13	0.34	0.26	0.098	0.1594

[1]University of Illinois Veterinary Diagnostic Laboratory Reference Ranges.
[2]Pooled standard error of the mean.

($P < 0.05$) ATTD of DM (81.8% vs. 83.2%) and NFE (90.7% vs. 92.9%) than those fed the control diet. The ATTD of OM, ether extract, crude ash, and crude fiber was not different ($P > 0.05$) among treatments. Kilburn et al. (2020) reported that the dogs fed extruded diets containing 8%, 16%, or 24% (as-is) banded cricket meal had a linear increase in fecal output (64.8 to 93.4 g/d as-is; 23.4 to 33.6 g/d DMB). Not surprisingly, the ATTD of DM (88.9% to 83.9%), OM (91.5% to 86.8%), CP (88.2% to 82.1%), fat (96.4% to 94.8%), and GE (92.4% to 88.3%) had a linear decrease ($P < 0.05$) with increased banded cricket meal inclusion. Lei et al. (2019) determined the ATTD of three dietary BSFL meal inclusion levels (0%, 1%, or 2% as-is). Even though ether extract digestibility was not affected ($P > 0.05$), linear increases ($P < 0.05$) in the ATTD of DM (71.97% to 75.21%) and nitrogen (73.16% to 78.51%) were observed. Finally, Freel et al. (2021) reported that no differences ($P > 0.05$) were observed in ATTD of DM, CP, fat, or GE for diets containing 0%, 5%, 10%, or 20% (as-is) BSFL meal or 2.5% or 5.0% (as-is) BSFL oil fed to adult dogs. Discrepancies among the studies reported above may be due to BSFL inclusion levels, the chitin content in BSFL ingredients tested, or other unknown variables affecting outcome measures.

Despite the interest that exists in using BSFL-based ingredient in pet foods, they have not been well tested in cats, especially in canned diets. Therefore, we investigated the effects of BSFL-ingredient containing canned diets on palatability, ATTD, fecal characteristics, serum metabolites, and hematology of adult cats in the current study. Experimental diets were formulated to contain a similar ingredient composition, similar nutrient and calorie content, and with practical inclusion levels of BSFL-based ingredients. Because CP content was lower and AHF, ash, and TDF concentrations were higher than expected in BSFL ingredients, the diets were not as consistent as desired. Because most of the BSFL ingredients had low moisture content, they provided some challenges in regard to diet formulation and production. Applying this to the pet food industry, these ingredients will be easier to incorporate into dry extruded diets. The TDF variance is likely due to the chitin content of BSFL-based ingredients (Finke, 2013; Bosch et al., 2014). Chitin (poly-N-acetyl-d-glucosamine) is an important component of BSFL formed in the cuticle layer during pupation and serves as an insoluble fiber in the diet of animals lacking endogenous chitinase activity (Roseland et al., 1985; Finke, 2013).

In the current studies, BSFL-containing diets were shown to have sufficient palatability. Food and calorie intake did not differ among treatments, but cats fed BSFL meal had higher ($P < 0.05$) fecal output and reduced nutrient digestibility. Paßlack and Zentek (2018) fed diets consisting of 22% or 35% of BSFL meal (as-is) to cats and reported that ATTD of CP (77.0% and 73.4%; $P < 0.05$) was reduced, while ATTD of crude fat (96.0% vs. 92.6%) was increased with increasing BSFL meal inclusion. As mentioned previously, the reduction in nutrient digestibility for cats fed BSFL meal in the current study may have been partly related to the chitin content of BSFL meal, which can negatively affect nutrient digestibility (Lei et al., 2019; Penazzi et al., 2021). Serum chemistry and complete blood count analysis were within reference ranges for adult cats. These results indicate that the treatments did not negatively affect any health outcomes. Similarly, Pezzali and Shoveller (2021) reported that serum chemistry and complete blood count parameters remained within reference ranges for adult cats fed a diet containing 4.6% BSFL meal for 21 d.

In conclusion, the addition of BSFL-based ingredients (BSFL meal, whole BSFL, and BSFL oil) had no detrimental effects on animal health or fecal quality. Inclusion of BSFL-based ingredients in canned diets improved palatability when compared with a poultry-based control diet. However, the inclusion of BSFL meal resulted in decreased ATTD of DM, OM, CP, and energy and the inclusion of whole BSFL reduced ATTD of OM, CP, and energy. Overall, dietary supplementation of BSFL ingredients were well accepted by healthy adult cats without any detrimental effects on fecal characteristics, serum chemistry, or hematology.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

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Conflict of interest statement

E.A.K. and A.M. are employed by EnviroFlight. All other authors have no conflicts of interest.

Literature Cited

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.

Barroso, F. G., M. J. Sánchez-Muros, M. Segura, E. Morote, A. Torres, R. Ramos, and J. L. Guil. 2017. Insects as food: enrichment of larvae of *Hermetia illucens* with omega 3 fatty acids by means of dietary modifications. *J. Food Compost. Anal.* 62:8–13. doi:10.1016/j.jfca.2017.04.008

Beynen, A. C. 2018. Insect-based petfood. *Creature Companion* 11:40–41.

Bosch, G., and K. S. Swanson. 2021. Effect of using insects as feed on animals: pet dogs and cats. *J. Insects Food Feed* 7:795–805. doi:10.3920/jiff2020.0084

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

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:skz363. doi:10.1093/jas/skz36

Do, S., L. Koutsos, P. L. Utterback, C. M. Parsons, M. R. C. de Godoy, and K. S. Swanson. 2021. 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. *J. Anim. Sci.* 99:1–10. doi:10.1093/jas/skab124

El-Wahab, A. A., L. Meyer, M. Kölln, B. Chuppava, V. Wilke, C. Visscher, and J. Kamphues. 2021. Insect larvae meal (*Hermetia illucens*) as a sustainable protein source of canine food and its impacts on nutrient digestibility and fecal quality. *Animals* 11:2525. doi:10.3390/ani11092525

Finke, M. D. 2013. Complete nutrient content of four species of feeder insects fed enhanced diets during growth. *Zoo Biol*. 34:554–564. doi:10.1002/zoo.21246

Firmansyah, M., and M. Y. Abduh. 2019. Production of protein hydrolysate containing antioxidant activity from *Hermetia illucens*. *Heliyon* 5:e02005. doi:10.1016/j. heliyon 2019:e02005

Fischer, C. L. 2020. Antimicrobial activity of host-derived lipids. *Antibiotics*. 9:75. doi:10.3390/antibiotics9020075

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

Kierończyk, B., M. Rawski, P. Pawełczyk, J. Różyńska, J. Golusik, Z. Mikołajczak, and D. Józefiak. 2018. Do insects smell attractive to dogs? a comparison of dog reactions to insects and commercial feed aromas – a preliminary study. *Ann. Anim. Sci*. 18:795–800. doi:10.2478/aoas-2018-0012

Kierończyk, B., J. Sypniewski, M. Rawski, W. Czekała, S. Świątkiewicz, and D. Józefiak. 2020. From waste to sustainable feed material: the effect of *Hermetia illucens* oil on the growth performance, nutrient digestibility, and gastrointestinal tract morphometry of broiler chickens. *Ann. Anim. Sci*. 20:157–177. doi:10.2478/aoas-2019-0066

Kilburn, L. R., A. T. Carlson, E. Lewis, and M. C. Rossoni Serao. 2020. Cricket (*Gryllodes sigillatus*) meal fed to healthy adult dogs does not affect general health and minimally impacts apparent total tract digestibility. *J. Anim. Sci*. 98:1–8. doi:10.1093/jas/skaa083

Kröger, S., C. Heide, and J. Zentek. 2020. Evaluation of an extruded diet for adult dogs containing larvae meal from the black soldier fly (*Hermetia illucens*). *Anim. Feed Sci. Technol*. 270:114699. doi:10.1016/j.anifeedsci.2020.114699

Lei, X. J., T. H. Kim, J. H. Park, and I. H. Kim. 2019. Evaluation of supplementation of defatted black soldier fly (*Hermetia illucens*) larvae meal in beagle dogs. *Ann. Anim. Sci*. 19:767–777. doi:10.2478/aoas-2019-0021

Liland, N. S., I. Biancarosa, P. Araujo, D. Biemans, C. G. Bruckner, R. Waagbø, B. E. Torstensen, and E. J. Lock. 2017. Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media. *PLoS One* 12:e0183188. doi:10.1371/journal.pone.0183188

Makkar, H. P. S., G. Tran, V. Heuze, and P. Ankers. 2014. State-of-the-art on use of insects as animal feed. *Anim. Feed Sci. Technol*. 197:1–33. doi:10.1016/j.anifeedsci.2014.07.008

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

Mouithys-Mickalad, A., E. Schmitt, M. Dalim, T. Franck, N. M. Tome, M. van Spankeren, D. Serteyn, and A. Paul. 2020. Black soldier fly (*Hermetia illucens*) larvae protein derivatives: potential to promote animal health. *Animals*. 10:941. doi:10.3390/ani10060941

Muzzarelli, R. A. A. 1977. *Chitin*. New York (NY): Pergamon Press, Inc.

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

Paßlack, N., and J. Zentek. 2018. Akzeptanz, verträglichkeit und scheinbare nährstoffverdaulichkeit von alleinfuttermitteln auf basis von *Hermetia-illucens*-Larvenmehl bei katzen (Acceptance, tolerance and apparent nutrient digestibility of complete diets based on larvae meal of *Hermetia illucens* in cats). *Tierärztliche Praxis Kleintiere* 46:213–221. doi:10.15654/TPK-180372

Penazzi, L., A. Schiavone, N. Russo, J. Nery, E. Valle, J. Madrid, S. Martinez, F. Hernandez, E. Pagani, and U. Ala. 2021. In vivo and in vitro digestibility of an extruded complete dog food containing black soldier fly (*Hermetia illucens*) larvae meal as protein source. *Front. Vet. Sci* 8:542. doi:10.3389/fvets.2021.653411

Pezzali, J. G., and A. K. Shoveller. 2021. Short communication: the effects of a semi-synthetic diet with inclusion of black soldier fly larvae meal on health parameters of healthy adult cats J. *Anim. Sci*. 99:skab290. doi:10.1093/jas/skab290

Prosky, L., N. G. Asp, I. Furda, J. W. DeVries, T. F. Schweizer, and B. F. Harland. 1985. Determination of total dietary fiber in foods and food products: collaborative study. *J. Assoc. Off. Anal. Chem*. 68:677–679.

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

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*. 95:379–386. doi:10.1603/0013-8746(2002)095[0379:SLHTOB]2.0.CO;2

Wang, Y. S., and M. Shelomi. 2017. Review of black soldier fly (*Hermetia illucens*) as animal feed and human food. *Foods* 6:91. doi:10.3390/foods6100091

Zheng, L., Y. Hou, W. Li, S. Yang, Q. Li, and Z. Yu. 2012. Biodiesel production from rice straw and restaurant waste employing black soldier fly assisted by microbes. *Energy* 47:225–229. doi:10.1016/j.energy.2012.09.006

Zhu, D., X. Huang, F. Tu, C. Wang, and F. Yang. 2020. Preparation, antioxidant activity evaluation, and identification of antioxidant peptide from black soldier fly (*Hermetia illucens* l.) larvae. *J. Food Biochem*. 44:e13186. doi:10.1111/jfbc.13186