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Chemical composition of selected insect meals and their effect on apparent total tract digestibility, fecal metabolites, and microbiota of adult cats fed insect-based retorted diets

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Abstract

Insect meals are novel and potentially sustainable protein sources. The objectives of this study were to determine the chemical composition and standardized amino acid digestibility using the cecectomized rooster model of three selected insect meals (i.e., speckled cockroach [**SC**], Madagascar hissing cockroach [**MC**], and superworm [**SW**]) and to determine the effects of these insect meals on food intake, apparent total tract digestibility (**ATTD**) of macronutrients, fecal scores, and metabolites of adult cats fed insect- or chicken-based retorted diets. This study consisted of a complete randomized design, with 28 adult cats randomly assigned to one of the four experimental retorted diets: Control (chicken-based diet), SC diet, MC diet, or SW diet. All animal procedures were approved by the University of Illinois Institutional Animal Care and Use Committee. All diets were formulated to be complete and balanced and meet or exceed the nutritional requirements of adult cats. The experimental period was 28 d, with the first 7 d allotted for diet adaptation. The total fecal collection was completed during the last 4 d of the experimental period. On day 21, a fresh fecal sample from each cat was collected for the determination of fecal metabolites and microbiota. Food was offered twice daily to maintain body weight and body condition score. Among the three selected insect meals evaluated, oleic acid, palmitic acid, linoleic acid, and stearic acid were the most prevalent fatty acids. Branched-chain amino acids and arginine were the most preponderant indispensable amino acids in these insect meals. ATTD of dry matter, organic matter, acid-hydrolyzed fat, and crude protein did not differ among treatments ($P > 0.05$), and all diets were well digested by the cats. Similarly, fecal scores did not differ among the treatments and were within ideal range. No differences ($P > 0.05$) in fecal metabolite concentrations or microbiota diversity were observed among cats fed different experimental diets; only a few genera from Firmicutes and Bacteroidota phyla differ ($P < 0.05$) in cats fed SW diet in contrast to other dietary treatments. In conclusion, the selected insect meals evaluated herein are rich in linoleic acid, an essential fatty acid for cats. Insect-based retorted diets led to comparable results to those achieved with a chicken-based retorted diet, suggesting that these novel protein sources might be adequate alternative ingredients in feline diets.

Lay Summary

Insect meals are novel and potentially sustainable protein sources. The objectives of this study were to determine the nutrient composition of speckled cockroach, Madagascar hissing cockroach, and superworm (**SW**) and to determine the effects of these insect meals on food intake, digestibility of macronutrients, fecal scores, metabolites, and microbiota of adult cats fed insect- or chicken-based wet pet foods. Among the three selected insect meals evaluated, oleic acid, palmitic acid, linoleic acid, and stearic acid were the most prevalent fatty acids. Branched-chain amino acids and arginine were the most preponderant indispensable amino acids in these insect meals. All diets were well digested by the cats with no differences observed on macronutrient digestibility. Similarly, fecal scores did not differ among the treatments and were within the ideal range. No differences in fecal metabolite concentrations were observed. Only a few genera from Firmicutes and Bacteroidota phyla differ in cats fed SW diet in contrast to other dietary treatments. Overall, the selected insect meals evaluated herein are rich in linoleic acid, an essential fatty acid for cats. Insect-based retorted diets led to comparable results to those achieved with a chicken-based retorted diet, suggesting that these novel protein sources might be adequate alternative ingredients in feline diets.

Key words: alternative protein, chemical composition, fatty acid, feline, insect, nutrient digestibility

Abbreviations: AAFCO, Association of American Feed Control Officials; AHF, acid-hydrolyzed fat; ATTD, apparent total tract digestibility; BCFA, branched-chain fatty acid; BHT, butylated hydroxytoluene; CBC, complete blood count; CP, crude protein; DM, dry matter; FAME, fatty acid methyl ether; FID, flame ionization detector; GE, gross energy; MC, Madagascar hissing cockroach (*Gromphadorhina portentosa*); OM, organic matter; OTU, operational taxonomic unit; PD, phylogenetic distance; SC, speckled cockroach (*Nauphoeta cinerea*); SCFA, short-chained fatty acid; SW, superworm (*Zophobas morio*)

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Introduction

Insect meals are considered high-quality protein sources, as they have been shown to have high protein and fat concentrations, and also good amino acid profile, which makes them potential ingredients for feline and canine foods (van Huis, 2013; Bosch et al., 2014). Moreover, the ratio of saturated/unsaturated fatty acids is also favorable for some insect species (van Huis, 2013). Additionally, insects can be sustainable protein sources with high feed efficiency when compared with livestock animals (Nakagaki and deFoliart, 1991). However, the nutrient composition of diets fed to insects can affect their feed conversation ratio and chemical composition. For example, Argentinian cockroach and black soldier fly are more efficient in the conversion of ingested foods of varying nutrient composition (14% to 30% and 17% to 24%, respectively) and on N conversion efficiency (51% to 87% and 43% to 52%, respectively) in contrast to yellow mealworm and house cricket (food conversion efficiency: 7% to 21% and 3% to 12%, respectively, and N conversion efficiency: 22% to 58% and 23% to 41%, respectively; Oonincx et al., 2015). In general, diets containing higher protein concentrations had positive effects, lowering feed conversion ratio and increasing feed conversion efficiency for these insects (Oonincx et al., 2015).

Despite increasing interest in the utilization of insect protein in pet foods, there is limited information on the chemical composition and nutritional adequacy and safety of these ingredients when incorporated in diets for companion animals. However, in recent decades, insect meals were already used as novel protein sources in the diets of some farm animals such as swine, fish, and poultry. From previous research studies, the specific inclusion of selected insect meals in diets of farm animals did not result in adverse effects on growth performance, digestibility of macronutrients, and product quality in poultry (0% to 15% black soldier fly larvae meal; Dabbou et al., 2018), swine (33% black soldier fly larvae meal; Newton et al., 1977), and aquaculture nutrition (36% fishmeal replaced by mealworm [*Tenebrio molitor* larvae] meal; Henry et al., 2018). Moreover, the chitin, which is a natural polysaccharide in the exoskeleton of insects, has similar properties of dietary fibers to promote fecal metabolites production and may also exert an immune-modulatory effect, even though mechanisms are still undetermined (Komi et al., 2017; Henry et al., 2018; Lei et al., 2019). However, most of the current literature on the evaluation of insects in animal nutrition has focused on determining the chemical composition and nutrition adequacy of black soldier fly larvae. Yet, different insects contain distinct amino acid and fatty acid profiles, with variable nutritional value and functionality. Therefore, the objectives of this study were to determine the chemical composition of three selected insect meals (i.e., speckled cockroach [**SC**], Madagascar hissing cockroach [**MC**], and superworm [**SW**]) and to determine the effects of these novel ingredients on food intake, macronutrient apparent total tract digestibility (**ATTD**), fecal scores, and metabolites of adult cats fed insect- or chicken-based retorted diets.

Materials and Methods

All animal care procedures were approved by the University of Illinois Animal Care and Use Committee (Protocol nos.: 19121 and 20131). All methods were performed in accordance with the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Precision-fed cecectomized rooster assay

A precision-fed cecectomized rooster assay was conducted using 12 cecectomized, single-comb White Leghorn roosters (4 roosters/treatment) according to Parsons (1985) to determine the standardized indispensable amino acid digestibility for each of the selected insect meals. The roosters were housed in a temperature-controlled room on a 16:8 (L:D) h schedule in wire-floored cages. After being fasted for 26 h, roosters were crop intubated with 24 g of the test substrate. Excreta were quantitatively collected after 48 h, freeze-dried as a single composite sample, and ground to a uniform particle size. Excreta were analyzed for amino acids according to AOAC (AOAC Official Method 982.30 E(a,b,c), chp. 45.3.05, 2006). Standardized amino acid digestibility was calculated using endogenous values that were derived across multiple roosters over several years according to Sibbald (1979).

Animals and diets

In this study, a total of 28 cats were used, with 18 spayed female and 10 neutered male adult domestic shorthair cats with average age of 2.1 ± 0.03 yr, mean body weight of 4.9 ± 0.8 kg, and mean body condition score of 5.4 ± 0.4 using a 9-point scale (Laflamme, 1997). All cats were housed in Edward R. Madigan Laboratory at the University of Illinois at Urbana-Champaign in a temperature- and light-controlled room, following a 14:10 (L:D) h schedule. Cats were individually housed during feeding (twice daily: 0800 to 1000 and 1500 to 1700 hours) and fecal collection periods but group housed during the remainder of the experimental period. Water was available ad libitum during all the times. During the study, body weight and body condition score were recorded weekly, and food intake was recorded daily. Cats were fed to maintain ideal body weight and body condition score throughout the study.

Three selected insect meal sources (i.e., SC, *Nauphoeta cinerea*; MC, *Gromphadorhina portentosa*; and SW, *Zophobas morio* larvae) were obtained from the Laboratory of Entomology of the Institute of Agricultural Sciences at the Federal University of Minas Gerais, Montes Carlos, Brazil. All insects were reared on a plant-based diet (i.e., soybean, corn, and wheat) and harvested by immersion in boiling water, followed by a 48-h drying period in a forced-air oven at 50 °C, and then ground using an electric screw meat grinder (Botini 1/3cv, model 1645, Grupo Botini, São Paulo, Brazil). The two species of cockroaches were harvested during the adult phase (60 d of age), whereas the superworm was harvested during the larval stage at approximately 90 d of age. The chemical composition of the insect meals is presented in **Table 1**. Four retorted diets were used in this experiment. All diets were formulated to have similar ingredient and chemical composition as well as to meet or exceed the nutrient profile for adult cats according to AAFCO (2018). In these experimental diets, most ingredients remained at a constant inclusion level among dietary treatments with the exception of the insect meal sources (i.e., SC, MC, and SW) that were added at 4% inclusion level at the expense of 3.5% chicken meal and 0.5% corn gluten meal of the control diet (**Table 2**). All experimental diets were manufactured at the

Table 1. Proximate analysis and indispensable amino acid and fatty acid composition of selected insect meal sources

Item	Insect meals[1]		
	SC meal	MC meal	SW meal
Dry matter, %	92.6	92.1	93.5
		Dry matter basis	
Organic matter, %	96.1	94.9	96.9
Crude protein, %	61.3	85.6	53.4
Acid-hydrolyzed fat, %	33.1	14.3	34.8
GE[2], kcal/g (measured)	6.6	5.6	6.8
Chitin, %	8.7	10.3	8.0
Indispensable amino acid, %			
Arginine	3.0	2.8	2.7
Histidine	1.5	1.6	1.6
Isoleucine	2.1	2.0	2.4
Leucine	3.5	3.4	3.6
Lysine	3.0	2.7	3.2
Methionine	0.9	0.7	0.6
Phenylalanine	2.2	2.1	2.2
Threonine	1.9	1.8	2.0
Tryptophan	0.5	0.4	0.8
Valine	3.6	3.9	3.4
Taurine	0.0	0.0	0.1
Fatty acid, ug/g			
Caprylic; C8:0	9.3	6.5	4,051.7
Capric; C10:0	13.7	5.3	673.7
Lauric; C12:0	177.4	43.8	127.7
Myristic; C14:0	1,626.8	402.0	1,684.2
Myristoleic; C14:1	240.6	26.7	13.2
Pentadecanoic; C15:0	179.3	74.9	302.4
Palmitic; C16:0	62,580.2	19,104.0	58,730.9
Palmitoleic; C16:1	12,878.7	3,432.9	1,321.1
Heptadecanoic; C17:0	347.4	229.3	663.1
Stearic; C18:0	14,638.6	6,473.1	21,653.7
Oleic; C18:1*n*9	113,351.8	46,352.6	78,562.5
Linoleic; C18:2*n*6	21,882.5	13,925.9	46,265.2
Chemical composition and amino acid and fatty acid profiles of selected insect meal sources			
Fatty acid, ug/g			
α-Linolenic; C18:3*n*3	2,060.0	848.7	1,739.1
Arachidic; C20:0	557.3	365.3	605.8
Arachidonic; C20:4*n*4	264.7	71.8	31.9
Eicosapentaenoic; C20:5*n*3[2]	n.d	n.d	n.d
Docosahexaenoic; C22:6*n*3[2]	n.d	n.d	n.d

[1]MC, Madagascar hissing cockroach (*Gromphadorhina portentosa*); SC, speckled cockroach (*Nauphoeta cinerea*); SW, superworm (*Zophobas morio* larvae).
[2]GE, gross energy; n.d., not detected.

Food Science and Human Nutrition Pilot Plant at University of Illinois at Urbana-Champaign. Briefly, ingredients for each dietary treatment were mixed by hand prior to being homogenized with opposite turning agitators in a jacketed steam kettle heated to 65.5 °C for 10 min. The pre-heated mixture then was added to cans (500 ± 5 g) and steam-flushed prior to sealing. Sealed cans then were statically retorted to reach an Fo value of 8 in an Allpax Gentle Motion Retort (Allpax, Covington, LA.). Wireless DataTrace temperature probes (Mesa Labs, Lakewood, CO) were used to calculate Fo values during the retort process.

Experimental design and sample collection

This experiment followed a complete randomized design, with the experimental period consisting of a 7-d adaptation period to the control diet, followed by a 21-d feeding

Table 2. Ingredient composition of retorted experimental diets

Item, % as-is basis	Treatments[1]			
	Control	SC diet	MC diet	SW diet
Water	39.49	39.49	39.49	39.49
Chicken	33.00	33.00	33.00	33.00
Steam	10.00	10.00	10.00	10.00
Rice flour	5.00	5.00	5.00	5.00
Corn gluten meal	**4.00**	**3.50**	**3.50**	**3.50**
Chicken liver	4.00	4.00	4.00	4.00
Chicken meal	**3.50**	**0.00**	**0.00**	**0.00**
Speckled cockroach; SC[1]	0.00	**4.00**	0.00	0.00
Madagascar hissing cockroach; MC[1]	0.00	0.00	**4.00**	0.00
Superworm; SW[1]	0.00	0.00	0.00	**4.00**
Guar gum	0.35	0.35	0.35	0.35
Carrageenan	0.15	0.15	0.15	0.15
Potassium chloride	0.13	0.13	0.13	0.13
Chelated mineral mix	0.10	0.10	0.10	0.10
Choline chloride 70%	0.09	0.09	0.09	0.09
Vitamin premix	0.08	0.08	0.08	0.08
Taurine	0.05	0.05	0.05	0.05
Salt, plain	0.05	0.05	0.05	0.05
Thiamine mononitrate	0.01	0.01	0.01	0.01

[1]MC, Madagascar hissing cockroach (*Gromphadorhina portentosa*); SC, speckled cockroach (*Nauphoeta cinerea*); SW, superworm (*Zophobas morio* larvae).

period in which cats were randomly assigned to one of the four experimental diets. At baseline (day 0) and on day 21, all cats were fasted overnight, and a total of 5 mL blood was collected from each cat into BD Vacutainer serum separator and Ethylenediaminetetraacetic acid (EDTA) tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) for serum chemistry and complete blood count (**CBC**) analyses, respectively. All samples were analyzed by the University of Illinois Veterinary School Diagnostics Laboratory (Urbana, IL).

During the last 4 d of the experimental period, the total fecal output was collected from all cats. During the same 4 d period, a fresh fecal sample from each cat was collected within 15 min of defecation. However, fresh samples from two cats were not obtained, and these cats were excluded from fecal metabolite and microbiota analyses. Fecal samples were scored using a 5-point scale: 1 = dry, hard pellets; 2 = firm, retains shape but pliable; 3 = soft and moist, but retains a shape; 4 = soft, unformed; and 5 = watery liquid; a score of 2 to 3 is considered ideal. Fecal pH was measured in fresh samples with a Denver Instrument AP10 pH meter (Denver Instrument, Bohemia, NY) with a Beckman electrode (Beckman Instruments, Inc., Fullerton, CA). These were then aliquoted out to be measured for dry matter (**DM**) and fermentative end-product concentrations, including short-chain fatty acids (**SCFA**s), branched-chain fatty acids (**BCFA**s), phenols, indoles, and ammonia. A 2-g aliquot of each fresh fecal sample was weighed in duplicate for DM and dried in a 105 °C oven for 48 h. A fecal aliquot of 3 g was weighed and placed in a 30-mL Nalgene bottle with 2 N hydrochloric acid and frozen at −20 °C until the analysis of SCFA, BCFA, and ammonia concentrations. Aliquots of 2 g were weighed in duplicates and placed in 16 mL plastic tubes for phenols and indoles and stored at −20 °C until analysis. Lastly, a portion of each fresh fecal sample was allocated for microbiota in 2 mL cryovials and stored at −80 °C until analysis. During the total fecal collection, all feces were weighed, scored, and stored in a −20 °C freezer until the analysis of ATTD of macronutrients.

Sample preparation and chemical analysis

The four retorted experimental diets were freeze-dried in an FTS Systems Dura-Dry MP freeze-dryer (SP Scientific, Warminster, PA). Fecal samples were dried in a 57 °C oven for approximately 7 d. Then, diet and fecal samples were ground in a Wiley Mill (model 4; Thomas Scientific, Swedesboro, NJ) through a 2-mm screen size in preparation for chemical analyses.

Insect meals, experimental retorted diets, and fecal samples were analyzed for DM, organic matter (**OM**), and ash according to AOAC (2006; methods 934.01 and 942.05). Crude protein (**CP**) was calculated based on total Nitrogen (**N**) concentration determined through Leco (TruMac N, Leco Corporation, St. Joseph, MI) according to AOAC (2006; method 992.15). Acid hydrolysis followed by ether extraction was used to determine the total lipid content according to the methods of the American Association of Cereal Chemists (1983) and Budde (1952). Gross energy (**GE**) was analyzed through the use of bomb calorimetry (Model 6200, Parr Instruments Co., Moline, IL). Complete amino acid profiles were determined for the three selected insect meals according to AOAC (2007). Chitin concentration of insect meals was determined according to Hornung and Stevenson (1971) and Ma and Zuazaga (1942) at the Pet Animal Nutrition Study Center in the Department of Animal Sciences at the Federal University of Lavras, Minas Gerais, Brazil. Briefly, 0.5 g of each insect meal was digested in 1 N NaOH solution for 5 h at 100 °C. After digestion, the solution plus sample was filtered, and the residue was washed five times with distilled water and once with methanol, followed by another distilled water rinse. Washed residues were dried in an oven at 37 °C for 24 h. Dried residues were then analyzed by micro-Kjeldahl methods as described by Ma and Zuazaga (1942).

SCFA and BCFA concentrations in fresh fecal samples were determined through the use of gas chromatography according to the method of Erwin et al. (1961) and Goodall and Byers (1978). Volatile fatty acid concentrations were determined using a Hewlett Packard (Hewlett Packard, Avondale, PA) Model 5890A gas chromatograph equipped with a flame ionization detector (**FID**) on a glass column (1.8 m × 4 mm i.d.) packed with GP 10% SP-1200/1% H_3PO_4 on 80/100 chromosorb W AW (Supelco, Bellefonte, PA). Nitrogen was the carrier gas and had a flow rate of 45 mL/min. The temperatures for the oven, injection port, and detector port were 125, 175, and 180 °C, respectively. The analyzed concentration from each set of duplicate tubes for acetate, propionate, butyrate, isobutyrate, isovalerate, valerate acid, and total SCFAs and BCFAs was averaged. Fecal phenol and indole concentrations were determined by gas chromatography according to the method of Flickinger et al. (2003) with

modifications. Phenol and indole analysis utilized a Thermo Scientific TRACE 1300 gas chromatograph coupled with an FID detector (Thermo Fisher Scientific, Waltham, MA). A 1-µL sample was injected at 220 °C, at splitless mode. A Nukol Supelcol column (60 m length, 0.32 mm diameter) with a film thickness of 0.25 µm was used to separate phenolic compounds. The oven temperature was initially 150 °C which was held for 1 min, and then it was increased at 25 °C per minute to 200 °C and held for 35 min. The internal standard used was 5-methylindole, and all samples were analyzed in duplicates. Ammonia concentrations were measured according to the method of Chaney and Marbach (1962).

Fatty acid analysis of selected insect meals and experimental diets

Fatty acid profiles of the three insect meals and the four experimental diets were determined according to Lepage and Roy (1986) and Masood et al. (2005) with modification in-house. Acetyl chloride, butylated hydroxytoluene (**BHT**), potassium carbonate, High-performance liquid chromatography(HPLC)-grade methanol, and hexane were purchased from Sigma-Aldrich (St. Louis, MO), while the internal standard (nonadecanoic acid, 19:0) and external fatty acids methyl ester standards were purchased from Supelco Sigma-Aldrich (St. Louis, MO). A sample size of 0.1 g was used for each duplicate. To prepare methanol–BHT solution with a ratio of 50 µg BHT/mL methanol to prevent the oxidation of fatty acids, add internal standard into methanol–BHT reagent at a concentration of 0.1 mg/mL. Then, 100 µL internal standard solution was mixed with the test substrates, and a 2 mL methanol–hexane (4:1, v/v) mixture was added. Tubes were vortexed and put on the ice. Then, 200 µL acetyl chloride was slowly added to the tubes and immediately placed under N gas. The samples were heated for 10 min at 100 °C, vortexed briefly, and heated for an additional 50 min. After heating, the tubes were placed on ice and allowed to cool and neutralized by adding 5 mL of a 6% Na_2CO_3 solution. The tubes were vortexed for 1 min and centrifuged at 2,300 × *g* at 4 °C for 3 min to separate the mixture into two phases. The upper organic phase was collected into a test tube, and the extraction was repeated once more by adding 0.5 mL of hexane, vortexing, and centrifuging for another 3 min at the same speed and temperature. The organic phase was collected again and combined with the first extraction. The combined extraction was evaporated under N to 300 µL, transferred to a gas chromatography vial with a 300-µL glass insert, and crimped under N gas for fatty acid methyl ether (**FAME**) analysis by gas chromatography. Thermo Scientific TRACE 1300 Gas Chromatograph coupled with FID was used for analyzing individual FAME. Samples (1 uL) were injected into gas chromatography (GC) and separated on a fused silica capillary column (SP-2560, 100 m length, 0.25 mm I.D., 0.2 um film thickness). The carrier gas was helium, and the flow rate was 20 cm/s, at a split ratio of 100:1. The temperature was at 140 °C initially for 5 min, then increased at 4 °C/min to a final temperature of 240 °C and held for 15 min. The temperatures for the injector and detector were 250 °C and 260 °C, respectively. The internal standard was nonadecanoic acid (C19:0, Nuchek Prep, Elysian, MN). Fatty acid methyl ester standards (Supelco 37 Component FAME Mix, Sigma-Aldrich) were used as an external standard to identify the fatty acid peaks in the samples by comparing with retention time.

Fecal microbiota

Total DNA extraction from fresh fecal samples was completed using a Mo-Bio PowerSoil kit (MO BIO Laboratories, Inc., Carlsbad, CA). Qubit 2.0 Fluorometer (Life Technologies, Grand Island, NY) was used to quantify DNA concentration prior to amplification and sequencing. A Fluidigm Access Array (Fluidigm Corporation, South San Francisco, CA), in combination with Roche High Fidelity Fast Start Kit (Roche, Indianapolis, IN), was used for the amplification of the *16S rRNA* gene. The primers 515F (5′-GTGYCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACNVGGGTWTCTAAT-3′), targeting a 292-bp fragment of the V4 region, were used for amplification (primers synthesized by IDT Corp., Coralville, IA; Caporaso et al., 2012). Fluidigm-specific primer, forward and reverse tags, was added in accordance with the Fluidigm protocol. Fragment Analyzer (Advanced Analytics, Ames, IA) was used to verify the quality of amplicons' regions and sizes. A DNA pool was generated through the combination of equimolar amounts of the amplicons from each sample. The pooled samples were selected by size on a 2% agarose E-gel (Life Technologies, Grand Island, NY) and extracted using a Qiagen gel purification kit (Qiagen, Valencia, CA). The pooled, size-selected, and cleaned products were then run on an Agilent Bioanalyzer in order to confirm appropriate profile and mean size. The Roy J. Carver Biotechnology Center at the University of Illinois performed Illumina sequencing on a MiSeq using v3 reagents (Illumina Inc., San Diego, CA). A FASTX-Toolkit (version 0.0.14) removed the Fluidigm tags. The analysis of sequences was completed using QIIME 2.0 (version 2020.6; Caporaso et al., 2010) and DADA2 (version 1.14; Callahan et al., 2016). The high-quality (quality value ≥ 20) sequence data, derived from the sequencing process, were de-multiplexed. An opened-reference operational taxonomic unit (**OTU**) clustered the sequences into OTUs, choosing against the SILVA 138 reference OTU database with a 97% similarity threshold (Quast et al., 2013). The OTUs observed fewer than two times (singletons), as well as OTUs with less than 0.01% of the total observation, were discarded. An average of 46,409 reads were obtained, with a total of 1,206,634 reads. The number of reads ranged from 38,614 to 56,225 per sample. Weighted and unweighted unique fraction metric (UniFrac) distances were performed by principal coordinate analysis (Lozupone et al., 2005).

Statistical analysis

Data were analyzed using SAS (SAS Institute Inc., version 9.4, Cary, NC), with a fixed effect of diet and a random effect of animal. Data normality was verified using the Univariate procedure. Differences among treatments were determined using a Fisher-protected least significant difference test with a Tukey adjustment to control for type-1 experiment-wise error. A probability of $P < 0.05$ was accepted as statistically significant, and reported pooled SEMs were determined according to the Mixed Models procedure.

Results and Discussion

Precision-fed cecectomized rooster assay

The standardized amino acid digestibility (**Table 3**) was calculated for the three insect meals based on the total excreta collected from the cecectomized roosters. The cecectomized

Table 3. Standardized amino acid digestibility of selected insect meals sources calculated using the precision-fed cecectomized rooster assay[1]

	Treatments[2]				
Amino acid	SC meal	MC meal	SW meal	Pooled SEM	*P*-value
Aspartate	92.6[a]	88.4[b]	93.9[a]	0.5329	0.0001
Threonine	91.1[a]	85.5[b]	94.1[a]	0.8795	0.0002
Serine	89.7[b]	83.6[c]	93.5[a]	0.8008	<0.0001
Glutamate	93.1[b]	90.4[c]	95.2[a]	0.4167	<0.0001
Proline	91.5[b]	79.8[c]	95.1[a]	0.7570	<0.0001
Glycine	84.4[a]	75.1[c]	62.7[b]	2.1537	0.0002
Alanine	91.1[b]	77.0[c]	94.3[a]	0.9090	<0.0001
Cyst(e)ine	80.7[a]	72.3[b]	81.4[a]	2.2746	0.0358
Valine	85.7[a]	73.8[b]	88.9[a]	1.0228	<0.0001
Methionine	93.7	94.7	93.2	0.4690	0.1317
Isoleucine	91.3[b]	87.9[c]	93.4[a]	0.4500	<0.0001
Leucine	93.1[a]	88.9[b]	94.4[a]	0.4747	<0.0001
Tyrosine	93.4[a]	82.2[b]	97.1[a]	1.3263	<0.0001
Phenylalanine	92.9[a]	89.4[b]	94.1[a]	0.5005	0.0002
Lysine	91.1[b]	90.8[b]	93.1[a]	0.3930	0.0069
Histidine	88.5[b]	76.4[c]	93.3[a]	0.7265	<0.0001
Arginine	94.8[a]	91.5[b]	95.4[a]	0.3826	0.0001
Tryptophan	93.4[a,b]	92.4[b]	95.3[a]	0.5874	0.0216

[1]*n* = 4 roosters per treatment.
[2]MC, Madagascar hissing cockroach (*Gromphadorhina portentosa*); SC, speckled cockroach (*Nauphoeta cinerea*); SW, superworm (*Zophobas morio* larvae).
[a–c]Means within a row with different superscripts are significantly different at $P < 0.05$.

rooster has proven to be an effective model for dogs, showing similar results in amino acid digestibility as ileal-cannulated dogs (Johnson et al., 1998). The use of cecectomized roosters can be utilized to accurately quantify the quality and digestibility of ingredients for use in pet foods. However, only the insect meals were used in this assay, and it should be noted that the use of additional heat processes commonly used in pet food production (i.e., extrusion and retort) can have an impact on nutrient bioavailability (van Rooijen et al., 2014).

All indispensable amino acids were well digested (>80%) in the three insect meals. The SW meal had consistently higher ($P < 0.05$) indispensable amino acid digestibility when compared with the MC meal. Histidine, isoleucine, and glutamate digestibilities were highest ($P < 0.05$) for the SW meal compared with the remaining two insect meals. Additionally, lysine digestibility was > 90% for all insect meals. As the first-limiting amino acid in most pet foods, the high digestibility of lysine in these ingredients is beneficial for adequate protein synthesis in the animal (Rosenberg, 1957). Glycine digestibility was different ($P < 0.05$) among all insect meals. Increased variation was seen in glycine digestibility due to glycine's role in the uric acid production pathway, a considerable component in rooster excreta (Corzo et al., 2009).

Do et al. (2020) analyzed the standard amino acid digestibility of black soldier fly larvae of varying ages using the precision-fed cecectomized rooster assay. The amino acid digestibility of the black soldier fly larvae was similar to the values measured in the current study, with the black soldier fly larvae aged 14 to 29 d tending to have higher (~90%) amino acid digestibility (Do et al., 2020). A different study measured the apparent ileal amino acid digestibility of mealworm larvae (*Tenebrio molitor*) and black soldier fly larvae (*Hermetia illucens*) meals in broiler chickens (de Marco et al., 2015). The overall apparent ileal digestibility of the insect meals was 86% for the mealworm larvae meal compared with 68% for the black soldier fly larvae (de Marco et al., 2015). Hall et al. (2018) determined the apparent ileal amino acid digestibility and true ileal amino acid digestibility of ground full-fat housefly (*Musca domestica*) larvae meal compared with fish meal. In that study, the apparent and true tryptophan digestibilities were higher in the insect meal (81% and 91%, respectively) compared with fishmeal (55% and 74%, respectively). The true ileal amino acid digestibility for the remaining amino acids ranged from 79% to 95% (Hall et al., 2018). Although variation exists in the composition and digestibility among different insect meal sources, the amino acid profiles of the insect meals studied are consistently well digested, making them viable, alternative protein sources.

Insect meal and experimental diet proximate analysis, food intake, and fecal characteristics

The three selected insect meals had similar DM (range: 92.1% to 93.5%) and OM (range: 94.9% to 96.9%) (**Table 1**). CP and acid-hydrolyzed fat (**AHF**) concentrations varied among insect meal sources. The MC meal had the highest concentration of CP (85.6%), in contrast to the SC meal (61.3%) and SW meal (53.4%). The latter, however, had the highest AHF concentration at 34.8%. The SC meal had a slightly lower AHF concentration at 33.1%, and the MC meal had the lowest concentration at 14.3%. GE also varied among insect meals but was reflective of the lipid content of each substrate. GE of SW meal (6.8 kcal/g) and SC meal (6.6 kcal/g) was comparable, whereas the MS meal had lower (5.6 kcal/g) GE content. Chitin concentration was the highest for MC meal (10.3%), with SC meal and SW meal having similar concentrations of 8.7% and 8.0%, respectively. The nutrient composition of edible insects varies substantially, depending on the species, origin, stage of life, and rearing feed (Finke and Oonincx, 2014).

To date, limited scientific information is available on the chemical composition and nutritional adequacy of insects in pet foods. Bosch et al. (2014) determined the proximate composition of 10 insect substrates; in general, all insects had high CP (47% to 71%) and fat (13% to 40%) concentrations. In the same study, three species of cockroaches (i.e., six spot cockroach [*Enblaberus distanti*], death's head cockroach [*Blaberus craniifer*], and Argentinean cockroach [*Blaptica dubia*]) and Morio worm larvae (*Zophobas morio*) were evaluated. CP for those three species of cockroaches ranged from 64% to 66%, which is similar to the CP concentration of the SC meal (61.3%) but lower than the MC meal (85.6%) in this study. Fat content among the three cockroach species evaluated by Bosch et al. (2014) was fairly consistent varying from 22% to 25%. In the present study, the AHF concentration for the MC meal was lower (14.3%) but higher for the SC meal (33.1%). Fat and CP concentration of SW meal (53.4% and 34.8%) differed from values previously reported of 47% and 40%, respectively (Bosch et al., 2014). A wide variation in amino acid composition among insect species and within species across life stages has been reported (Ramos-Elorduy et al., 2002; Rumpold and Schluter, 2013; Bosch et

al., 2014; Ghosh et al., 2017; Do et al., 2020). Higher indispensable amino acid concentrations were reported by Bosch et al. (2014) for Morio worm larvae and the three species of cockroaches analyzed in that study in contrast with our findings. Ghosh et al. (2017) reported similar (i.e., arginine, isoleucine, leucine, threonine, and valine) or slightly lower (i.e., lysine, methionine, and phenylalanine) concentrations of indispensable amino acids of larvae of different species of edible beetles and crickets. In general, black soldier fly larvae at 0 d and 18 to 29 d of age had comparable indispensable amino acid concentrations with the selected insects evaluated in this study, although lower concentrations of indispensable amino acids were reported at 11 and 14 d of age (Do et al., 2020). Discrepancies observed in the chemical composition can be related to intrinsic differences in the nutrient composition among insect species, feed that these insects were reared off, as well as age, harvesting, and processing methods.

Fatty acid profile (ug/g on a DM basis) of insect meals varied (**Table 1**). Among the three selected insect meals evaluated herein, the most predominant fatty acids were: oleic acid (113,352 to 46,353 ug/g), palmitic acid (62,580 to 19,104 ug/g), linoleic acid (46,265 to 13,926 ug/g), and stearic acid (21,654 to 6,473 ug/g). The SW meal had higher levels of caprylic (4,052 ug/g) and capric (674 ug/g) acids compared with SC meal (9.3 and 13.7 ug/g, respectively) or MC meal (6.5 and 5.3 ug/g, respectively). Myristic acid concentration was similar between SC (1,627 ug/g) and SW (1,684 ug/g) meals but lower for MC meal (402.0 ug/g). A greater concentration of palmitoleic acid was present in the SC meal (12,879 ug/g) in contrast with MC meal (3,433 ug/g) and SW meal (1,321 ug/g). Similarly, α-linolenic acid concentration was higher in the SC meal (2,060 ug/g) in comparison with SW meal (1,739 ug/g) and MC meal (848.7 ug/g). Arachidonic acid was present at low concentration in the three selected insect meals varying from 31.9 to 264.7 ug/g. Eicosapentaenoic and docosahexaenoic acids were not present at detectable concentrations in these substrates.

While a few studies have examined the proximate and amino acid composition of a wide variety of insect species, including cockroaches (Bosch et al., 2014), different sources of worms and beetles (Bosch et al., 2014; Ghosh et al., 2017), and black soldier fly larvae and pupae (Bosch et al., 2014; Liu et al., 2017; Do et al., 2020), the fatty acid profile of most edible insect sources still largely unknown despite these ingredients containing copious levels of lipids in addition to being protein-rich ingredients. Most insects can biosynthesize palmitic, stearic, and oleic acids (Paul et al., 2017; Benzertiha et al., 2019). Our findings are in agreement with previous literature that reported palmitic, stearic, oleic, and linoleic acids being among the most prevalent fatty acids in a variety of insect substrates. Linoleic acid is an essential fatty acid for dogs and cats (NRC, 2006). Dietary supplementation of this fatty acid has been shown to improve skin and coat scores in dogs, as this fatty acid seems not to be extensively metabolized and desaturated, with a large proportion being deposited in the skin and fur of pet animals (Marsh et al., 2000; Fu et al., 2001).

The chemical composition of the retorted experimental diets is presented in **Table 4**. Experimental diets had comparable concentrations of macronutrients; DM and OM concentrations varied from 20.4% to 24.4% and 90.9% to 93.1%, respectively. The SC and SW diets had lower CP concentrations but higher concentrations of AHF compared with control or MC diets. GE of experimental diets, on average, was 6.1 kcal/g. Fatty acid profiles of experimental diets are also presented in **Table 4**. In general, less variation was observed in the fatty acids profile of the experimental diets than measured in the insect meals. This was expected as the inclusion of insect meal corresponded to only 4% of the ingredient composition of these diets, with chicken and chicken liver comprising the main ingredients in these diets and were kept at a constant inclusion level. The four most predominant fatty acids among the experimental diets were: oleic acid, palmitic acid, linoleic acid, and stearic acid. Overall, the variation in chemical composition among the experimental diets was likely due to the fixed rate of inclusion of selected insect meals (4%) added at the expense of 3.5% of chicken meal and 0.5% of corn gluten meal (**Table 2**).

Daily food intake (g/d, as is or g/d, dry matter basis [DMB]) did not ($P > 0.05$) differ among dietary treatments (**Table 5**). However, a wide variation in food intake was observed among cats with this behavior being independent of dietary treatment. Throughout the experimental period, some cats consistently

Table 4. Chemical composition and fatty acid profile of retorted experimental feline diets containing selected insect meal sources

Item	Treatments[1]			
	Control	SC diet	MC diet	SW diet
Dry matter, %	24.4	22.3	22.3	20.4
	%, Dry matter basis			
Organic matter, %	93.1	93.1	90.9	91.3
Crude protein, %	43.6	39.5	44.4	39.1
Acid-hydrolyzed fat, %	23.6	26.7	24.0	27.4
GE[2], kcal/g (measured)	5.9	6.1	6.1	6.3
Fatty acid, ug/g				
Caprylic; C8:0	38.2	32.9	33.0	962.5
Capric; C10:0	23.2	18.0	12.1	158.7
Lauric; C12:0	75.3	98.3	70.8	89.1
Myristic; C14:0	1,244.6	1,269.3	1,032.8	1,428.1
Myristoleic; C14:1	343.6	319.7	262.3	291.6
Pentadecanoic; C15:0	154.7	155.3	133.3	195.3
Palmitic; C16:0	52,864.1	52,404.4	45,034.3	57,038.1
Palmitoleic; C16:1	12,248.3	12,100.2	9,852.1	10,607.1
Heptadecanoic; C17:0	295.8	292.1	279.0	382.7
Stearic; C18:0	14,371.3	13,792.1	13,799.4	15,989.8
Oleic; C18:1*n*9	77,462.6	79,804.6	66,777.5	81,150.4
Linoleic; C18:2*n*6	43,263.9	36,482.2	33,751.1	43,460.8
α-Linolenic; C18:3*n*3	1,909.3	1,803.7	1,464.9	1,845.2
Arachidic; C20:0	212.9	280.0	271.1	295.3
Arachidonic; C20:4*n*4	1,795.2	1,319.4	2,621.8	1,168.8
Eicosapentaenoic; C20:5*n*3[2]	n.d.	n.d.	n.d.	n.d.
Docosahexaenoic; C22:6*n*3	123.0	102.8	152.3	105.8

[1]MC, Madagascar hissing cockroach (*Gromphadorhina portentosa*); SC, speckled cockroach (*Nauphoeta cinerea*); SW, superworm (*Zophobas morio* larvae).
[2]GE, gross energy; n.d., not detected.

Table 5. Food intake and fecal characteristics of cats fed retorted experimental diets containing selected insect meal sources

Item	Treatments[1]					
	Control	SC diet	MC diet	SW diet	SEM	*P*-value
Food intake, g/d (as-is)	168.3	212.9	156.6	162.3	18.655	0.1722
Food intake, g/d (dry matter [DM] basis)	41.1	47.4	34.9	33.0	4.265	0.1132
Fecal characteristics/output						
Fecal score[2]	2.2	2.3	1.9	2.2	0.161	0.2647
Fecal pH	6.4	6.8	7.0	6.6	0.202	0.2449
Fecal output, as-is basis, g/d	16.9	20.0	11.4	12.7	3.320	0.2792
Fecal output, DM basis, g/d	6.0	6.3	4.1	4.3	0.867	0.1971

[1]MC, Madagascar hissing cockroach (*Gromphadorhina portentosa*); SC, speckled cockroach (*Nauphoeta cinerea*); SW, superworm (*Zophobas morio* larvae).
[2]Fecal scores: 1 = day, hard pellets; 2 = firm, retains shape but pliable; 3 = soft and moist, but retains a shape; 4 = soft, unformed; and 5 = watery liquid.

consumed 100% of their daily food ration, whereas others had variable daily consumption ranging from 30% to 100% of the daily food ration. Cats can be peculiar about food flavor profile, texture, shape, and temperature (Zaghini and Biagi, 2005; German and Heath, 2015). Therefore, it is possible that these cats were demonstrating a neophobic behavior toward the retorted diets, as these animals had been strictly fed extruded diets during their lifespan prior to this study. While cats may show neophilia toward novel diets, it seems that food preference for novel diets (also known as the monotony effect) is more prevalent in free-ranging cats than cats raised exclusively on nutritionally complete diets (Church et al., 1996). Despite intermittent inappetence, all cats remained healthy based on the cats' daily behavior (only observational) and serum metabolites and CBC results (data not shown). Serum chemistry did not differ among dietary treatments, and all values, with exception of creatinine and glucose concentrations, were within the reference range provided by the University of Illinois Veterinary Diagnostics Laboratory. The creatinine concentration of all treatments was approximately 1.7 mg/dL, which is slightly higher than its reference range (0.4 to 1.6 mg/dL). However, the concentration of creatinine was the same on days 0 (baseline) and 21 (end of experimental period). Similarly, serum glucose concentrations among cats fed experimental diets were above the reference range on both collection periods without any significant differences among treatments. Transient hyperglycemia observed in these cats is attributed to sedative used prior to blood collection.

Fecal scores were not affected ($P > 0.05$) by dietary treatment and were within the ideal range using a 5-point scale, ranging, on average, from 1.9 to 2.3 for cats fed the MC and SC diets, respectively. Fecal pH ranged from 6.4 for cats fed the control diet to 7.0 for cats fed the MC diet. Fecal output (g/d) on an as-is or DM basis also did not differ ($P > 0.05$) among dietary treatments (**Table 5**). Ideal fecal scores have also been reported in kittens fed raw diets (Hamper et al., 2016). Kerr et al. (2013) reported a similar average fecal score of 2.6, using the same 5-point scale as of the present study, when African wildcats (*Felis silvestris lybica*) were fed a retorted feline diet.

ATTD of macronutrients, digestible energy, and fecal fermentative end products

The inclusion of selected insect meals at the expense of chicken meal and corn gluten meal resulted in comparable ($P > 0.05$) ATTD of macronutrients and digestible energy of retorted diets when fed for adult cats for a 21-d period (**Table 6**). On average, DM ATTD varied between 86.5% and 88.1%. Similarly, the ATTD of OM, CP, and AHF was high and between 88.9% to 90.6%, 86.3% to 89.4%, and 90.1% to 92.3%, respectively. Digestible energy did not differ among dietary treatments and, on average, had a coefficient of digestibility of 90% across dietary treatments, resulting in a caloric content of 5.5 kcal/g on average. Unfortunately, limited scientific data on in vivo macronutrient ATTD of pet foods containing insects are available. In vitro OM and N digestibility of Morio worm of 91% and 92%, respectively, was reported by Bosch et al. (2014). Those same authors reported lower in vitro OM and N digestibility values for six spot cockroach and death's head cockroach (78% and 76% and 79% and 78%, respectively) but similar in vitro OM (84%) and N (84%) digestibility for Argentinean cockroach in comparison with our findings. Previous research in our laboratory demonstrated that when these insect meals were added at 7.5% and 15% inclusion levels in extruded diets of adult cats and dogs, macronutrient ATTD was not negatively affected (Lisenko et al., 2018a, 2018b).

The fecal metabolites analyzed included ammonia, phenols, indoles, SCFAs, and BCFAs (**Table 7**). Fecal metabolites of cats fed diets containing selected insect meals did not differ ($P > 0.05$) from cats fed the control diet. Generally, SCFAs are considered to be beneficial to gut health, and SCFA production is associated with microbial hindgut saccharolytic fermentation (Gross et al., 2019). Borrelli et al. (2017) found when replacing soybean meal (23.5%) with defatted *H. illucens* larvae meal (17%), the ceca SCFA concentration in these birds increased (acetate:10.8 mmol/L in insect diet and 6.9 mmol/L in soybean diet, $P < 0.001$; propionate: 5.8 mmol/L in insect diet and 3.0 mmol/L in soybean diet, $P < 0.001$; butyrate: 4.4 mmol/L in insect diet and 1.5 mmol/L in soybean diet, $P < 0.001$).

Fecal microbial communities

The fecal microbial composition at the phylum level (**Table 8**) showed that the abundant phyla included Actinobacteriota, Bacteroidota, Campilobacterota, Desulfobacterota, Firmicutes, Fusobacteriota, and Proteobacteria. The phyla composition was not different ($P > 0.05$) among the treatment groups. The most abundant phyla were Bacteroidota (ranging from 16.2% in cats fed SC to 22.3% in cats fed

Table 6. Apparent total tract macronutrient and energy digestibility of cats fed retorted experimental diets containing selected insect meal sources

Item	Treatments[1]					
	Control	SC diet	MC diet	SW diet	SEM	*P*-value
Dry matter, %	87.88	86.92	88.12	86.49	1.334	0.803
	%, DM basis					
Organic matter, %	90.64	89.22	89.88	88.86	1.085	0.656
Crude protein, %	89.43	86.64	88.62	86.31	1.333	0.294
Acid-hydrolyzed fat, %	90.14	90.33	92.27	90.53	1.260	0.625
Digestible energy, %	90.14	89.23	90.49	89.35	1.140	0.841

[1]MC, Madagascar hissing cockroach (*Gromphadorhina portentosa*); SC, speckled cockroach (*Nauphoeta cinerea*); SW, superworm, *Zophobas morio* larvae.

Table 7. Fecal fermentative end products of cats fed retorted experimental diets containing selected insect meal sources

	Treatments[1]					
Item, µmole/g (DM basis)	Control	SC diet	MC diet	SW diet	SEM	*P*-value
Ammonia	166.2	161.2	142.3	187.5	20.43	0.7343
Phenols & indoles						
Total phenols/indoles	4.4	3.2	2.9	4.6	0.60	0.9715
Phenol	0.1	0.2	0.1	0.1	0.06	0.4443
Indole	4.2	3.0	2.8	4.5	0.59	0.2757
SCFAs[2]						
Acetate	170.4	207.9	117.7	218.3	36.65	0.3119
Propionate	68.6	92.5	40.0	94.0	17.35	0.1106
Butyrate	41.6	43.5	31.0	49.0	6.20	0.6377
BCFAs[2]						
Isobutyrate	8.9	8.4	9.0	10.5	1.33	0.3460
Isovalerate	15.7	13.5	13.5	18.9	2.24	0.4333
Valerate	10.4	11.1	8.8	10.7	1.27	0.8580

[1]MC, Madagascar hissing cockroach (*Gromphadorhina portentosa*); SC, speckled cockroach (*Nauphoeta cinerea*); SW, superworm (*Zophobas morio* larvae).
[2]SCFA, short-chain fatty acid; BCFA, branched-chain fatty acid.

SW) and Firmicutes (ranging from 67.7% in cats fed SW to 73.1% in cats fed SC). In general, it is not uncommon for a combination of Firmicutes and Bacteroidetes phyla to comprise approximately 98% of the mammalian gut microbiota (Thomas et al., 2011). Over 40 different microbial families were identified (**Table 8**) and demonstrated variable relative abundance among treatments. The most abundant families included Coriobacteriaceae, Bacteroidaceae, Tannerellaceae, Erysipelotrichaceae, Lachnospiraceae, and Ruminococcaceae. A higher relative abundance ($P < 0.05$) of Bacteroidaceae was observed in cats fed SW (13.6%) compared with cats fed SC (6.7%) and MC (7.0%). Although unknown, the higher relative abundance of Bacteroidaceae may be due to the high phosphorus content of superworms, which was analyzed by Finke (2015) to be 2,090 mg/kg. One of the primary enzyme categories produced by Bacteroidaceae is phosphatases (Keudell and Goldberg, 1970). Additionally, cats fed SW had higher relative abundances ($P < 0.05$) of Tannerellaceae and Butyricicoccaceae (2.3% and 1.4%, respectively) compared with cats fed SC (1.2% and 0.4%, respectively). The fecal microbial composition at the genera level is reported in **Table 9**. While over 63 genera were identified, only 4 of those genera were different ($P < 0.05$) among dietary treatments. Cats fed SC had consistently lower ($P < 0.05$) relative abundances of *Bacteroides* (6.7%), *Parabacteroides* (1.2%), *Butyricicoccus* (0.2%), and *Oscillibacter* (0.2%) compared with cats fed SW. *Oscillibacter* has been associated with ammonium and indole production in humans (Amaretti et al., 2019). Although not significantly different ($P > 0.05$), cats fed SW had numerically higher fecal ammonia and indole concentrations compared with SC. Previous research evaluating the effects of nutritional interventions on feline and canine gut microbiota revealed that feeding periods as short as 14 d were sufficient to shift gut microbiota and (or) their corresponding metabolites (Detweiler et al., 2019; Nogueira et al., 2019; Reilly et al., 2021). The subtle fecal microbial changes observed herein could be due to the inclusion level of insect meal at 4% of the experimental diets. This was necessary as it is challenging to add larger quantities of dry ingredients to wet pet food formulations.

As an emerging alternative protein source, minimal studies have focused on the effect of insect meals on the fecal microbiota of companion animals. One study analyzed the cecal microbiota of laying hens fed black soldier fly (*H. illucens*) larvae compared with hens fed a soybean meal diet using

Table 8. Relative abundance of bacterial phyla and families of cats fed retorted diets containing selected insect meals

Phylum	Family	Dietary treatment[1]				*P*-value
		Control	SC diet	MC diet	SW diet	
Actinobacteriota		4.5	3.7	4.1	3.8	0.8067
	Coriobacteriaceae	3.6	2.9	2.9	2.5	0.3048
	Eggerthellaceae	0.8	0.8	1.0	1.3	0.3845
Bacteroidota		17.3	16.2	17.3	22.3	0.2996
	Bacteroidaceae	9.2[a,b]	6.7[b]	7.0[b]	13.6[a]	0.0049
	Tannerellaceae	1.4[a,b]	1.2[b]	1.3[a,b]	2.3[a]	0.0279
	Prevotellaceae	5.1	6.9	7.8	4.0	0.3448
Campilobacterota			0.7	0.5	0.8	0.8694
	Campylobacteraceae	0.7	0.8	0.5	0.8	0.8705
Desulfobacterota			0.9	1.0	1.1	0.9537
	Desulfovibrionaceae	0.9	0.8	1.0	1.1	0.9532
Firmicutes		72.3	73.1	72.0	67.7	0.5461
	Butyricicoccaceae	0.8[a,b]	0.4[b]	0.6[a,b]	1.4[a]	0.0137
	Erysipelotrichaceae	11.7	11.3	11.2	11.2	0.9945
	Lachnospiraceae	28.3	26.9	29.9	27.5	0.7289
	Oscillospiraceae	3.3	2.9	2.9	3.7	0.5718
	Ruminococcaceae	5.8	6.7	6.4	6.1	0.9024
Fusobacteriota		1.5	2.6	3.2	2.3	0.2885
	Fusobacteriaceae	1.5	2.3	3.2	2.3	0.2879
Proteobacteria		2.9	2.7	2.0	2.1	0.7515
	Enterobacteriaceae	1.4	1.1	0.8	0.6	0.5724

[1]MC, Madagascar hissing cockroach (*Gromphadorhina portentosa*); SC, speckled cockroach (*Nauphoeta cinerea*); SW, superworm (*Zophobas morio* larvae).
[a,b]Means within a row with different superscript letters are different ($P < 0.05$).

Table 9. Relative abundance of bacterial genera of cats fed retorted diets containing selected insect meals

Phylum	Genera	Dietary treatment[1]				*P*-value
		Control	SC diet	MC diet	SW diet	
Bacteroidota	*Bacteroides*	9.2[b]	6.7[b]	7.0[b]	13.6[a]	0.0049
	Parabacteroides	1.4[a,b]	1.2[b]	1.3[a,b]	2.3[a]	0.0278
Firmicutes	*Butyricicoccus*	0.7[a,b]	0.2[b]	0.5[a,b]	1.1[a]	0.0070
	Oscillibacter	0.4[a,b]	0.2[b]	0.4[a,b]	0.8[a]	0.0507

[1]MC, Madagascar hissing cockroach (*Gromphadorhina portentosa*); SC, speckled cockroach (*Nauphoeta cinerea*); SW, superworm (*Zophobas morio* larvae).
[a,b]Means within a row with different superscript letters are different ($P < 0.05$).

16S sequencing (Borrelli et al., 2017). Firmicutes (49%), Bacteroidetes (32%), and Proteobacteria (8%) were the most abundant phyla, with the hens fed *H. illucens* having higher relative abundances of Elusimicrobia, Lentisphaerae, and Cyanobacteria than in hens fed soybean meal (Borrelli et al., 2017). At the species level, hens fed *H. illucens* had higher relative abundances of *Bacteroides plebeius, Bacteroides salanitronis*, and *Oscillospira flavonifractor plautii* (Borrelli et al., 2017). In chickens fed 7.5% mealworm (*Tenebrio molitor*) meal, 16S sequencing demonstrated that Bacteroidetes was the predominant phyla in the ceca, followed by Firmicutes and Proteobacteria (Biasato et al., 2018). A higher relative abundance of Firmicutes was analyzed in chickens fed mealworm meal than in chickens fed corn gluten meal, with *Clostridium, Ruminococcus*, *Oscillospira*, and *Faecalibacterium* as the predominant genera (Biasato et al., 2018). In humans consuming 25 g/d of a dried cricket powder, the most abundant phyla were Bacteroidetes and Firmicutes (comprising 90% of the analyzed sequences) compared with humans who did not consume the cricket powder, with no differences at the genera level (Stull et al., 2018). Increasing levels of whole cricket meal (0% to 24%) in extruded diets for adult healthy dogs also led to modest fecal microbial shifts in these animals, with an increased relative abundance of *Catenibacterium*, Lachnospiraceae [*Ruminococcus*], and *Faecalitalea*, and decreased abundance of Bacteroides, *Faecalibacterium*, and Lachnospiraceae NK4A136 group (Jarett et al., 2019). Similar to our findings,

Firmicutes and Bacteroidetes were also the most abundant microbial phyla, whereas Coriobacteriaceae, Bacteroidaceae, Erysipelotrichaceae, Lachnospiraceae, and Ruminococcaceae were among the most abundant families in dogs fed extruded diets with or without increasing levels of whole cricket meal (Jarett et al., 2019).

The α-diversity (**Figure 1**) was measured using Faith's phylogenetic distance. Species evenness was similar among cats fed the different dietary treatments. In humans consuming cricket powder, the α-diversity, measured using the Shannon diversity index, showed no differences in species evenness (Stull et al., 2018). In contrast, Borrelli et al. (2017) measured α-diversity using the Shannon diversity index and found that diversity within microbial populations of laying hens fed *H. illucens* larvae was higher than in hens fed a soybean meal diet. The β-diversity based on weighted and unweighted UniFrac analysis is shown in **Figures 2 and 3**. For both weighted and unweighted, the fecal microbial community abundance was similar between cats fed the selected insect meals. In the Stull et al. (2018) study, β-diversity was measured using Bray–Curtis Dissimilarity and demonstrated that there were no differences in microbial populations between humans consuming cricket powder compared with those who did not. Similarly, Jarett et al. (2019) reported no differences in α- or β-diversities of adult healthy dogs fed a chicken-based extruded diet in contrast with dogs fed up to 24% of dietary protein content coming from whole cricket meal.

Conclusions

Overall, the three selected insect meals evaluated in this study are lipid and protein-rich ingredients, containing high concentrations of linoleic acid, an essential fatty acid for cats with potential benefits for the skin and coat health, and high concentrations of branched-chain amino acids and arginine. The inclusion of 4% of these insect meals in replacement of chicken meal and corn gluten meal in retorted diets for adult cats resulted in no negative effects on the ATTD of macronutrients, fecal scores, metabolites, microbiota, and overall

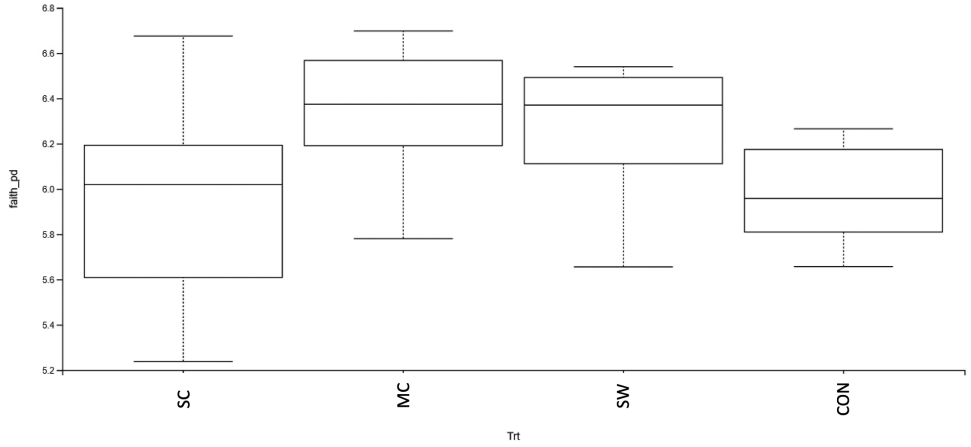

Figure 1. Alpha-diversity analysis of fecal microbial communities, measured by Faith's phylogenetic diversity (PD), of cats fed diets containing selected insect meal sources. Abbreviations: CON, Control; MC, Madagascar hissing cockroach (*Gromphadorhina portentosa*); SC, speckled cockroach (*Nauphoeta cinerea*); SW, superworm (*Zophobas morio* larvae).

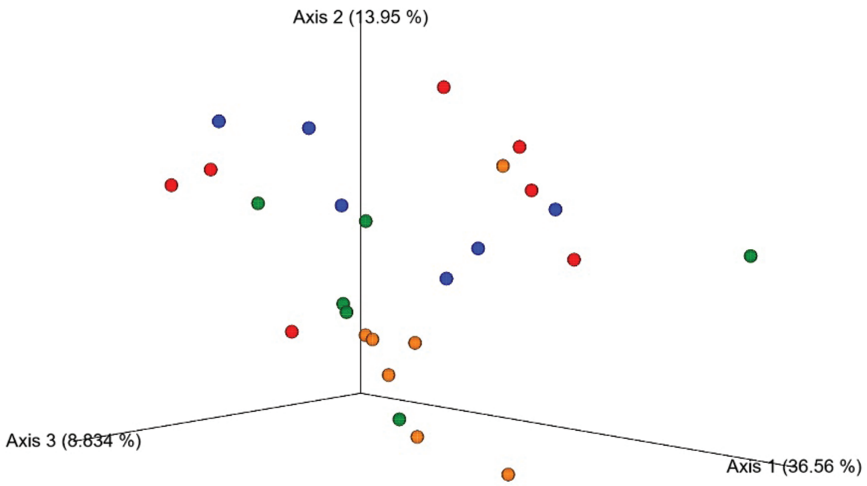

Figure 2. Principal coordinated plots of weighted UNIFRAC distances of fecal microbial communities of cats fed diets containing selected insect meal sources. Abbreviations: CON, Control (green); MC, Madagascar hissing cockroach (*Gromphadorhina portentosa*; blue); SC, speckled cockroach (*Nauphoeta cinerea*; red); SW, superworm (*Zophobas morio* larvae; orange).

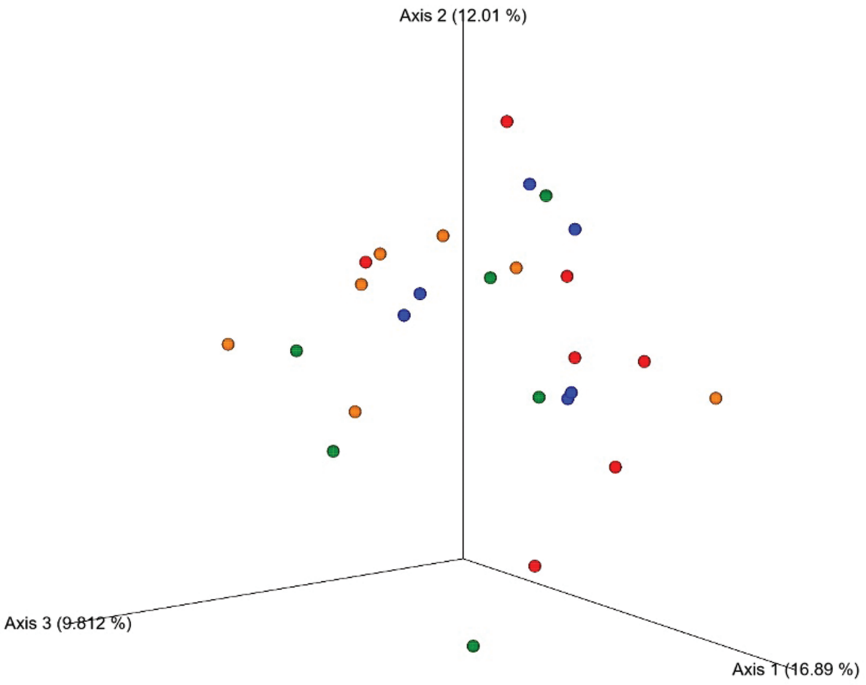

Figure 3. Principal coordinated plots of unweighted UNIFRAC distances of fecal microbial communities of cats fed diets containing selected insect meal sources. Abbreviations: CON, Control (green); MC, Madagascar hissing cockroach (*Gromphadorhina portentosa*; blue); SC, speckled cockroach (*Nauphoeta cinerea*; red); SW, superworm (*Zophobas morio* larvae; orange).

animal health. Therefore, the data gathered herein suggest that these selected insect meals are adequate alternative ingredients for feline diets and comparable to traditional protein sources used in pet food. Further research should evaluate the potential beneficial effects of these ingredients in the skin and coat of pet animals.

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Conflict of interest statement

The authors have no conflict of interest to declare. L.L. is an employee of Simmons Pet Food, Inc., company that provided partial financial support for this work.

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