

## COMPANION ANIMAL NUTRITION

# Cricket (*Gryllobes sigillatus*) meal fed to healthy adult dogs does not affect general health and minimally impacts apparent total tract digestibility

Logan R. Kilburn,<sup>†</sup> Anne T. Carlson,<sup>‡</sup> Elizabeth Lewis,<sup>||</sup> and Mariana C. Rossoni Serao<sup>†,1</sup>

<sup>†</sup>Department of Animal Science, Iowa State University, Ames, IA 50011, <sup>‡</sup>Jiminy's LLC, Berkeley, CA 94704, <sup>||</sup>NutraSteward, Bridge Innovation Center, Pembroke Dock SA72 6UN, UK

<sup>1</sup>Corresponding author: [mcrserao@iastate.edu](mailto:mcrserao@iastate.edu)

## Abstract

Insects can serve as a novel high-quality protein source for pet foods. However, there is an absence of research investigating the use of insects in pet food. The study objective was to evaluate the apparent total tract digestibility and possible health effects of diets containing graded levels of cricket (*Gryllobes sigillatus*) meal fed to healthy adult dogs. Thirty-two adult Beagles were randomly assigned to one of four dietary treatments: 0%, 8%, 16%, or 24% cricket meal. Dogs were fed their respective diet for a total of 29 d with a 6-d collection phase. Fecal samples were collected daily during the collection phase to measure total fecal output as well as apparent total tract digestibility for dry matter (DM), organic matter, crude protein, fat, total dietary fiber, and gross energy. Blood samples were taken prior to the study and on day 29 for hematology and chemistry profiles. Data were analyzed in a mixed model including the fixed effects of diet and sex. Total fecal output increased on both an as-is ( $P = 0.030$ ) and DM basis ( $P = 0.024$ ). The apparent total tract digestibility of each nutrient decreased ( $P < 0.001$ ) with the increasing level of cricket meal inclusion. All blood values remained within desired reference intervals indicating healthy dogs. Slight fluctuations in blood urea nitrogen ( $P = 0.037$ ) and hemoglobin ( $P = 0.044$ ) levels were observed but were not considered of biological significance. Even with the decrease in digestibility with the inclusion of cricket meal, diets remained highly digestible at greater than 80% total apparent digestibility. In conclusion, crickets were demonstrated to be an acceptable ingredient for dog diets.

**Key words:** crickets, dogs, protein

## Introduction

The pet food industry is constantly evolving due to consumer demand. To be successful, pet food companies must discover ways to create novel products to meet these demands. The use of insects as an ingredient in pet food could be the next trend in the pet food industry. There is already interest in insects for food application, where it could serve as a more sustainable protein source than meat. Insects require fewer resources and

emit fewer greenhouse gas emissions compared with livestock raised for food production (Ooninx and de Boer, 2012). Insects can also be grown on food waste, contributing to circular economies (Salomone et al., 2017). Van Huis et al. (2013) reported that compared with conventional livestock at 40% to 60% insects have a greater edible component at 80%, which leads to less unused products. Furthermore, studies in livestock support their suitability to partially or completely replace conventional protein sources, such as fishmeal and soybean meal

**Abbreviations**

AOAC	Association of Official Analytical Chemists
BUN	blood urea nitrogen
CP	crude protein
DM	dry matter
EDTA	Ethylenediaminetetraacetic acid
GE	gross energy
OM	organic matter
SEM	standard error of the means
TDF	total dietary fiber

(Makkar et al., 2014). This may fuel the development of large-scale rearing of insects in which the pet food industry could take advantage of to meet consumer demands.

A variety of cricket species have been shown to have a high nutritious value. Previous studies have shown that crickets contain 58 to 78% crude protein (CP) and up to 18% fat on a dry matter (DM) basis (DeFoliart et al., 1982; Nakagaki et al., 1987; Finke et al., 1989; Barker et al., 1998; Wang et al., 2005; Moreki et al., 2012). According to the swine NRC (2012), the CP content of cricket meal is comparable to fishmeal and soy protein concentrate. In addition, the amino acid profile of crickets and fishmeal is similar (Finke, 2002; Wang et al., 2005). Of note, the nutrient composition varies depending on an insect's life stage, diet, and origin.

Only a few studies have investigated the use of crickets in diets fed to monogastric animals. Miech et al. (2017) reported the apparent nutrient digestibility of diets containing crickets to be higher or similar to that of fishmeal when fed to piglets. For example, the digestibility of crude fiber for the diet containing whole crickets was 48% while that of fishmeal was 31% (Miech et al., 2017). In addition, ground Mormon crickets have been reported to be a suitable protein source for rats (Finke et al., 1987).

The current study is one of the first long-term feeding trials in which general blood parameters of animals were analyzed after consuming diets containing cricket meal. For the evaluation of novel foods, it is important to have such studies as they provide information about nutritional adequacy beyond fecal nutrient digestibility. Therefore, the objective of this study was to determine the apparent digestibility and any possible health effects resulting from diets containing graded levels of cricket meal fed to healthy adult dogs.

**Materials and Methods**

The study was conducted at Summit Ridge Farms in Susquehanna, PA, and was approved by the Summit Ridge Farms' Institutional Animal Care and Use Committee on June 28, 2018.

**Animals and housing**

Thirty-two Beagles (16 males and 16 females),  $4.75 \pm 2.5$  yr old with an initial body weight of  $9.69 \pm 1.9$  kg (mean  $\pm$  SD), were enrolled in this study. All animals were healthy, passing a veterinary physical examination and baseline hematology and clinical chemistry screening prior to the start of the study. Dogs were also of optimal weight and body condition. Dogs were housed in individual runs with 16 ft<sup>2</sup> of raised floor space in a temperature-controlled facility (15 to 24°C) kept on a 12-h light/12-h dark cycle. Grated floors allowed fecal output

to fall through to prevent coprophagy. Dogs were socialized and provided daily interaction with other dogs and staff. Dogs did not have outside access during the study to prevent the consumption of foreign material.

**Diets and feeding**

A total of four diets, formulated to meet current Association of American Feed Control Officials' guidelines for dogs, were used containing increasing levels of cricket meal: 0% (control), 8%, 16%, or 24% cricket meal (Table 1). Crickets were raised under closed and controlled conditions and in accordance with the requirements for the production of food-grade insects. The cricket meal added to the diets was produced from banded crickets (*Grylodes sigillatus*) raised on a modified chicken feed until maturity (~35 to 40 d). Reared crickets were frozen before being washed, roasted at 93.3 °C for 6 h, and milled into a fine meal (425  $\mu$ m). The nutrient composition of the cricket meal specifically used in this study is provided in Table 2. Raw ingredients were purchased from and ground with a hammer mill using a 3/64-inch screen by Fairview Mills (Seneca, KS). Diets were processed using an X115 single screw extruder and dried using a Wenger Enhanced Sanitary Dryer. Diet samples were stored for future analyses.

Dogs were randomly assigned to one of four dietary treatments in a complete randomized design with eight dogs per treatment (four males and four females). Each treatment was fed for a total of 29 d, using a 23-d adaption phase followed by a 6-d collection phase. This study duration is internationally recognized as suitable for novel proteins as highlighted by European Food Safety Authority's guidance for feed additives and for novel biomasses. Dogs were individually fed their respective diet once a day at 0700 hours and given the day for consumption. Feeding amounts were adjusted weekly to maintain body weight but were not adjusted during the collection period. Daily feed intake and any orts were recorded for each dog throughout the experiment. Water was provided ad libitum using an automatic watering system throughout the study.

**Sample collection**

Total fecal output was collected daily during the collection phase and averaged to determine daily fecal output (g as-is/d). Feces collected during the 6-d collection period were pooled, homogenized, and stored at 4 °C for each dog before the nutrient analysis. Additional fecal collections were performed on days 14 and 28 for microbial analysis (as reported in Jarett et al., 2019). Fecal scores were recorded at least three times a day during the collection phase according to the following scale: 0 = none, 1 = watery diarrhea, 1.5 = diarrhea, 2 = moist, no form, 2.5 = moist, some form, 3 = moist, formed, 3.5 = well-formed, sticky, 4 = well-formed, 4.5 = hard, dry, and 5 = hard, dry, crumbly.

A 5-mL blood sample was collected from each dog via jugular venipuncture at baseline and on day 29 of the study for hematology and chemistry profiles. The sample was split into two collection tubes. Serum tubes were spun in a refrigerated centrifuge for 15 min at 3,000 rpm after being allowed to clot. Ethylenediaminetetraacetic acid (EDTA) tubes were placed on a rocker for 15 min to allow the blood to adequately mix with the anticoagulant.

**Laboratory analyses**

Nutrient composition of the cricket meal was provided by the supplier. DM, CP, crude fat, and ash analyses of the cricket meal

**Table 1.** Ingredient composition of diets

Ingredient, %	Cricket meal			
	0%	8%	16%	24%
Corn	37.57	37.57	37.57	37.57
Chicken meal	21.69	14.46	7.22	0.00
Cricket meal	0.00	8.00	16.00	24.00
Brewers rice	15.00	15.00	15.00	15.00
Chicken fat	7.69	7.06	6.43	5.80
Corn gluten meal	6.00	6.00	6.00	6.00
Dried beet pulp	3.50	3.50	3.50	3.50
Corn starch	2.58	1.73	0.92	0.09
Natural flavor	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.80	2.16	2.47	2.83
Calcium carbonate	0.69	1.05	1.42	1.74
Salt	0.50	0.50	0.50	0.50
Potassium chloride	0.40	0.40	0.40	0.40
Fish oil	0.25	0.25	0.25	0.25
Choline chloride 60%	0.10	0.10	0.10	0.10
LANI vitamin premix <sup>1</sup>	0.10	0.10	0.10	0.10
LANI trace mineral premix <sup>2</sup>	0.05	0.05	0.05	0.05
LANI organic trace mineral premix <sup>3</sup>	0.01	0.01	0.01	0.01
LANI Naturox Plus <sup>4</sup>	0.04	0.04	0.04	0.04

<sup>1</sup>LANI vitamin premix (pea fiber, calcium carbonate, vitamin E, niacin, thiamine mononitrate, D-calcium pantothenate, vitamin A, sunflower oil, pyridoxine hydrochloride, riboflavin, vitamin D3, biotin, vitamin B12, and folic acid).

<sup>2</sup>LANI trace mineral premix (calcium carbonate, zinc sulfate, ferrous sulfate, copper sulfate, mineral oil, manganous oxide, sodium selenite, and calcium iodate).

<sup>3</sup>LANI organic trace mineral premix (zinc methionine complex, calcium carbonate, zinc sulfate, iron proteinate, ferrous sulfate, copper proteinate, copper sulfate, manganese proteinate, sunflower oil, manganous oxide, sodium selenite, calcium iodate, and ethylenediamine dihydroiodide).

<sup>4</sup>LANI Naturox Plus (amorphous silicon dioxide, citric acid, natural mixed tocopherols, vegetable oil, and rosemary extract).

were performed using the Association of Official Analytical Chemists (AOAC) methods 950.46A, 990.03, 922.06, and 923.03, respectively. Fiber was analyzed using the American Oil Chemists' Society Ba 6a-05 method and amino acid compositions were analyzed using the AA USDA MSS2 (1993) method.

Total fecal collections and dietary treatments were analyzed for DM, organic matter (OM), CP, crude fat, total dietary fiber (TDF), and gross energy (GE). All chemical analyses were conducted in the Comparative Nutrition Laboratory at Iowa State University (Ames, IA). Fecal samples and dietary subsamples were dried at 65 °C in a forced air-drying oven and ground in order to pass through a 1.0-mm screen in a Wiley grinder (Model ED-5, Thomas Scientific Inc., Swedesboro, NJ). Diet and fecal samples were analyzed for DM (AOAC 934.01) and OM (AOAC 942.05). Nitrogen was determined using a LECO Nitrogen Analyzer (AOAC 992.15; model TruMacN; LECO Corporation; St. Joseph, MI). An EDTA sample of 9.56% nitrogen was used as the standard for calibration. Crude protein was estimated by multiplying the analyzed nitrogen content by 6.25. Crude fat was determined via acid hydrolysis and hexane extraction (AOAC 960.39). GE was determined via bomb calorimetry (model 6200; Parr Instrument Co.; Moline, IL) with benzoic acid (6,318 kcal GE/kg; Parr Instrument Co.) used as the standard for calibration. TDF was analyzed at Midwest Laboratories (Omaha, NE). Blood samples were packaged and sent priority-overnight for the analysis to Antech Diagnostics (Memphis, TN) for hematology (Siemens Advia 120) and clinical chemistry (Beckman Coulter AU5800).

**Table 2.** Nutrient composition of the cricket meal included in diets (provided by the supplier)

Nutrient	% DM
DM	98.23
Crude protein	67.76
Crude fat	21.64
Ash	4.79
Crude fiber	7.51
Alanine	5.40
Arginine	4.12
Aspartic acid	6.67
Cystine	ND <sup>1</sup>
Glutamic acid	8.73
Glycine	3.13
Histidine	1.58
Isoleucine	2.80
Leucine	4.96
Lysine	3.35
Methionine	1.16
Phenylalanine	3.48
Serine	3.48
Taurine	ND
Threonine	2.76
Tryptophan	ND
Tyrosine	3.47
Valine	3.99
Amino acid recovery <sup>2</sup>	87.19

<sup>1</sup>Not determined.

<sup>2</sup>Amino acid recovery = sum of amino acids/ % crude protein.

### Apparent total tract digestibility calculation

Apparent total tract macronutrient and energy digestibility were determined using chemical composition data from diet and fecal samples and feed intake/fecal output records. Apparent total tract macronutrient and GE digestibility were calculated using the following equation:

$$\text{Apparent digestibility (\%)} = \left( \frac{\text{intake} - \text{fecal output}}{\text{intake}} \right) \times 100$$

### Statistical analysis

Normality of residuals were tested using PROC UNIVARIATE. Data were analyzed in a mixed model including the fixed effects of diet and sex (PROC MIXED, Version 9.4, SAS Inst., Cary, NC). A diagonal covariance structure was used with initial body weight as a covariate for analysis of body weights recorded during the duration of the study and baseline blood values as a covariate for final blood parameters. Differences between diets were determined using least squared means. A probability of  $P < 0.05$  was considered statistically significant and standard error of the means (SEM) were determined. Orthogonal contrasts to determine linear, quadratic, or cubic relationships were also analyzed.

## Results and Discussion

### Diet and fecal chemical analyses

Nutrient concentrations of the diets ranged for DM (92.0% to 93.4%), OM (92.9% to 93.6%), CP (26.1% to 28.0%), fat (13.1% to 14.2%), and GE (4,891 to 4,932kcal/kg) (Table 3). TDF steadily increased from 1.92% (control) to 3.86% (24% cricket meal). Replacement of chicken meal with cricket meal increased DM, OM, CP, fat, GE, and TDF in the diets. Comparing the control with the 24% diet, the fiber content was approximately 2× greater. The increased fiber content of the diets may be explained by chitin, a component of an insect's exoskeleton, which is recovered in fiber analyses (Koutsos et al., 2019). Crickets have been reported to contain 7% to 9% chitin on a DM basis (Finke, 2002; Wang et al., 2005), which monogastric animals are unable to digest (Ngoan et al., 2000; Ngoan and Lindberg, 2001). The level of cricket meal inclusion in canine diets might be dictated by the higher concentration of TDF in the diet due to its possible impact on fecal characteristics and digestibility.

### Feed intake and fecal characteristics

Feed intake and fecal characteristics are presented in Table 4. There were no significant differences for as fed ( $P = 0.385$ ) or DM ( $P = 0.380$ ) intake or mean body weight ( $P = 0.827$ ) among

**Table 3.** Analyzed chemical composition of diets, % DM

Item	Cricket meal			
	0%	8%	16%	24%
DM (as-is)	92.0	92.4	93.0	93.5
Moisture (as-is)	8.04	7.56	7.00	6.55
OM	93.2	92.9	93.5	93.6
Ash	6.78	7.14	6.51	6.45
Crude protein	26.1	26.4	27.8	28.0
Fat	13.4	13.1	14.2	13.7
TDF	1.92	2.44	3.48	3.86
GE, kcal/kg DM	4,901	4,891	4,930	4,932

**Table 4.** Average feed intake, fecal output, fecal score, fecal pH, apparent total tract macronutrient, and energy digestibility

Item	Cricket meal				SEM	P-value			
	0%	8%	16%	24%		Treatment	Linear	Quadratic	Cubic
<b>Intake</b>									
Feed intake, g AF/d	231	193	227	222	16.81	0.385	0.944	0.322	0.155
Feed intake, g DM/d	213	178	211	208	15.61	0.380	0.818	0.327	0.155
GE intake, kcal/d	1,043	873	1,039	1,023	76.78	0.354	0.758	0.322	0.143
<b>Output</b>									
Fecal output, g as-is/d	64.8 <sup>a</sup>	66.2 <sup>a</sup>	70.3 <sup>a</sup>	93.4 <sup>b</sup>	7.16	0.030	0.009	0.142	0.618
Fecal output, g DM/d	23.4 <sup>a</sup>	24.0 <sup>a</sup>	26.4 <sup>a</sup>	33.6 <sup>b</sup>	2.44	0.024	0.005	0.181	0.773
Fecal score	3.40	3.44	3.47	3.43	0.03	0.336	0.324	0.136	0.682
Fecal pH	6.53	6.36	6.19	6.18	0.14	0.232	0.053	0.545	0.792
<b>Apparent digestibility</b>									
DM, %	88.9 <sup>a</sup>	86.5 <sup>b</sup>	87.3 <sup>ab</sup>	83.9 <sup>c</sup>	0.68	<0.001	<0.001	0.475	0.025
OM, %	91.5 <sup>a</sup>	89.4 <sup>b</sup>	90.0 <sup>ab</sup>	86.8 <sup>c</sup>	0.54	<0.001	<0.001	0.320	0.013
Crude Protein, %	88.2 <sup>a</sup>	84.8 <sup>b</sup>	86.0 <sup>b</sup>	82.1 <sup>c</sup>	0.74	<0.001	<0.001	0.715	0.007
Fat, %	96.4 <sup>a</sup>	95.7 <sup>b</sup>	96.0 <sup>ab</sup>	94.8 <sup>c</sup>	0.22	<0.001	<0.001	0.360	0.013
TDF, %	57.5 <sup>a</sup>	43.7 <sup>b</sup>	61.3 <sup>a</sup>	46.3 <sup>b</sup>	2.81	<0.001	0.214	0.828	<0.001
GE, %	92.4 <sup>a</sup>	90.4 <sup>b</sup>	90.8 <sup>b</sup>	88.3 <sup>c</sup>	0.49	<0.001	<0.001	0.628	0.024

<sup>a-c</sup>Means within a row lacking a common superscript letter are different ( $P < 0.05$ ).

**Table 5.** Serum chemistry analysis of dogs fed diets containing graded levels of cricket meal

Item	Cricket meal				SEM	Treatment	P-value			Reference interval <sup>1</sup>
	0%	8%	16%	24%			Linear	Quadratic	Cubic	
BUN, mg/dL	11.7 <sup>a</sup>	11.3 <sup>a</sup>	13.2 <sup>b</sup>	12.2 <sup>a</sup>	0.47	0.037	0.118	0.464	0.020	6.0 to 31.0
Creatinine, mg/dL	0.56	0.60	0.60	0.58	0.02	0.605	0.582	0.212	0.979	0.5 to 1.6
BUN/Creat Ratio	21.1	19.2	22.0	21.6	1.08	0.273	0.379	0.487	0.108	4.0 to 27.0
Glucose, mg/dL	81.5	84.7	84.2	81.3	2.43	0.758	0.922	0.300	0.902	70.0 to 138.0
Total Protein, g/dL	6.26	6.17	6.29	6.21	0.09	0.811	0.973	0.927	0.339	5.0 to 7.4
Albumin, g/dL	3.25	3.29	3.26	3.27	0.05	0.953	0.815	0.754	0.689	2.7 to 4.4
Globulin, g/dL	3.00	2.89	2.99	2.98	0.08	0.688	0.968	0.448	0.352	1.6 to 3.6
Albumin/Globulin Ratio	1.13	1.17	1.11	1.12	0.04	0.699	0.617	0.611	0.348	0.8 to 2.0
Alkaline Phosphatase, U/L	72.6	59.0	49.5	60.8	7.42	0.203	0.188	0.105	0.622	5.0 to 131.0
Aspartate aminotransferase, U/L	26.4	24.5	27.1	24.9	1.31	0.470	0.764	0.915	0.126	15.0 to 66.0
alanine transaminase, U/L	43.8	40.5	37.4	33.1	3.28	0.148	0.024	0.894	0.929	12.0 to 118.0
gamma-glutamyl transferase, U/L	5.29	4.50	5.09	4.37	0.29	0.109	0.109	0.902	0.052	1.0 to 12.0
creatine phosphokinase, U/L	105	101	142	123	15.35	0.228	0.178	0.625	0.130	59.0 to 895.0
Bilirubin, mg/dL	0.16	0.17	0.14	0.15	0.02	0.840	0.596	0.839	0.493	0.1 to 0.3
Cholesterol, mg/dL	172	174	167	168	7.16	0.878	0.573	0.901	0.593	93.0 to 324.0
Triglycerides, mg/dL	62.2	55.0	60.4	56.8	4.18	0.620	0.575	0.678	0.253	29.0 to 291.0
Sodium, mEq/L	146.8	145.7	146.2	146.5	0.37	0.231	0.831	0.093	0.281	139.0 to 154.0
Potassium, mEq/L	4.38	4.19	4.31	4.17	0.09	0.399	0.254	0.798	0.199	3.6 to 5.5
Chloride, mEq/L	112.3	112.8	113.5	112.9	0.57	0.489	0.342	0.309	0.575	102.0 to 120.0
Calcium, mg/dL	9.91	9.90	9.86	9.83	0.08	0.884	0.430	0.968	0.928	8.9 to 11.4
Phosphorus, mg/dL	3.79	3.41	3.76	3.42	0.19	0.315	0.423	0.934	0.092	2.5 to 6.0
Magnesium, mEq/L	1.48	1.55	1.56	1.58	0.03	0.131	0.030	0.466	0.654	1.5 to 2.5

<sup>1</sup>Reference intervals are laboratory specific.

<sup>a-c</sup>Means within a row lacking a common superscript letter are different (P < 0.05).



Table 6. Hematology profile of dogs fed diets containing graded levels of cricket meal

Item	Cricket meal				SEM	Treatment	P-value			Reference interval <sup>1</sup>
	0%	8%	16%	24%			Linear	Quadratic	Cubic	
WBC, 10 <sup>3</sup> /mm <sup>3</sup>	7.64	7.36	7.76	7.68	0.57	0.958	0.863	0.637	0.841	4.0 to 15.5
RBC, 10 <sup>6</sup> /mm <sup>3</sup>	6.83	6.77	6.61	6.51	0.11	0.197	0.901	0.769	0.036	4.8 to 9.3
Hemoglobin, g/dL	15.7 <sup>a</sup>	15.5 <sup>ab</sup>	15.1 <sup>bc</sup>	14.9 <sup>c</sup>	0.22	0.044	0.856	0.540	0.006	12.1 to 20.3
Hematocrit, %	51.1	49.6	48.4	48.0	0.81	0.060	0.533	0.908	0.009	36.0 to 60.0
Mean corpuscular volume, $\mu\text{m}^3$	74.4	73.6	73.1	74.0	1.05	0.856	0.441	0.833	0.762	58.0 to 79.0
Mean corpuscular hemoglobin, $\mu\text{g}$	23.0	23.0	22.8	23.0	0.22	0.845	0.693	0.432	0.899	19.0 to 28.0
Mean corpuscular hemoglobin concentration, g/dL	30.8	31.1	31.4	31.3	0.25	0.350	0.362	0.872	0.124	30.0 to 38.0
Platelets, 10 <sup>3</sup> /mm <sup>3</sup>	272	264	317	285	14.42	0.078	0.410	0.032	0.179	170.0 to 400.0
Absolute Monocytes	444	341	384	392	64.45	0.772	0.418	0.517	0.712	0.0 to 840.0
Absolute Eosinophils	199	264	306	311	33.96	0.119	0.390	0.931	0.023	0.0 to 1,200.0
Absolute Basophils	0.00	0.00	0.00	0.00	0.00	1.000	1.000	1.000	1.000	0.0 to 150.0
Absolute Bands	0.00	0.00	0.00	0.00	0.00	1.000	1.000	1.000	1.000	0.0 to 300.0
Absolute Polys	5,177	4,654	4,975	5,148	451.14	0.843	0.471	0.617	0.910	2,060.0 to 10,600.0
Absolute Lymphocytes	1,903	2,010	2,058	1,885	102.97	0.578	0.182	0.723	0.991	690.0 to 4,500.0

<sup>1</sup>Reference intervals are laboratory specific.<sup>a-c</sup>Means within a row lacking a common superscript letter are different ( $P < 0.05$ ).

treatments. However, there were significant differences for fecal output on both an as-is ( $P = 0.030$ ) and DM ( $P = 0.024$ ) basis when comparing treatments. In addition, fecal output followed a linear relationship with cricket inclusion ( $P \leq 0.009$ ). The increased fecal output may be explained by the increase in dietary fiber. Previous studies have shown an increase in wet fecal weight with the increase in dietary fiber (Bueno et al., 1981; McPherson-Kay, 1987; Fahey et al., 1990; Cole et al., 1999). This result may be due to the “bulking effect” of fiber and appears to be most strongly associated with insoluble fiber sources which are poorly fermentable and have a good water-binding capacity (Diez et al., 1998). Further research is needed to access the water-binding capacity of chitin. Typically, with the increase in wet fecal weight, the DM output is not altered, meaning the main contributor is increased water content in the stool. However, in this study, the DM output was also significantly impacted. In vitro fermentation of undigested insect fractions was found to be negligible in non-adapted dogs, though there might be fermentation (and production of short-chain fatty acids) when the dog microbiota adapts to the fractions (Bosch et al., 2016). Jarett et al. (2019) previously published from this study that microbial communities among fecal samples minimally differed among treatments. This indicates that the increase in fecal output with increased cricket inclusion was not due to increased microbial abundance. Short-chain fatty acid concentrations were not directly measured in this study, but production rates have been reported to increase with dietary fiber inclusion (Sunvold et al., 1995). In addition, the decreased digestibility with the increase in fiber may have led to the increased DM fecal output. Notably, the fecal DM content remained low at 26.4% with the 16% cricket meal inclusion possibly indicating an optimum inclusion level.

Fecal scores were maintained at acceptable levels with an average of 3.4 or 3.5 for each treatment. Fecal pH also did not differ among treatments ( $P = 0.232$ ), suggesting the addition of chitin did not alter fermentation yielding short-chain fatty acids (Bosch et al., 2016). In addition, the maintenance of fecal pH could indicate that the change in protein source did not impact protein fermentation. Although fecal output was altered, other fecal characteristics were maintained as levels of cricket meal increased.

### Apparent total tract digestibility

Apparent digestibility ranged for 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%) from the control to the 24% cricket meal diet (Table 4). The apparent digestibility for fiber was much lower ranging from 57.5% to 46.3%. The low level of fiber digestibility is to be expected due to its ability to resist hydrolysis by endogenous enzymes. Most dietary fiber passes to the large intestine undigested where it can then be fermented by microbes (NRC, 2006). Each nutrient digestibility had significant differences among treatments ( $P < 0.001$ ). Linear ( $P < 0.001$ ) and cubic ( $P < 0.05$ ) relationships were observed in DM, fat, GE, OM, and CP digestibility with the increase in cricket meal. Fiber digestibility only presented a cubic relationship ( $P < 0.001$ ). Fahey et al. (1990) showed a similar range for fiber digestibility as well as a cubic relationship when testing increasing levels of beet pulp, 5% to 14% TDF, in diets fed to dogs. Cubic relationships could indicate an optimum inclusion level. Cole et al. (1999) reported a linear decrease in DM, OM, and GE digestibility with an increase in soybean hulls in dog diets containing 3% to 9% TDF. Likewise, chitin has previously been implicated as a factor in the reduced digestibility of insects in livestock and aquaculture (Dumas et al., 2018). Concerns regarding chitin and the negative impact on digestibility are complicated by a lack of analytical methods (Koutsos et al.,

2019). Interestingly, Bosch et al. (2014) reported the in vitro OM digestibility of house crickets to be 88% which was similar when compared with poultry meat meal at 85.8%. Of note, this in vitro study reported maximal digestibility in line with the true ileal digestibility approach while apparent fecal digestibility tends to underestimate true digestibility. Nonetheless, the apparent fecal DM digestibility of each treatment in this study is still greater than 80%, which is comparable to commercially manufactured dog foods (Castrillo et al., 2001). Notably, crickets in this study were roasted and ground; other processing methods may influence results (Poelaert et al., 2018).

### Blood panels

Blood results and reference intervals for healthy dogs are presented in Table 5 and Table 6. Blood samples were analyzed to determine any fluctuations among treatments and to monitor health status. Blood urea nitrogen (BUN;  $P = 0.037$ ) and hemoglobin ( $P = 0.044$ ) levels were the only blood parameters with significant results among treatments. BUN presented a significant cubic ( $P = 0.020$ ) relationship with the increase in cricket inclusion. As a result of amino acid oxidation and urea cycle activity, urea is produced by the liver and is carried by the blood to the kidney for excretion. Even though diets were formulated to be isonitrogenous, protein levels of the diets numerically increased with increased cricket meal. Therefore, the increase in dietary protein could have led to fluctuations in BUN levels (Hosten, 1990). Hemoglobin presented a linear decrease with the increase of cricket meal ( $P = 0.006$ ). A possible speculation of the decrease in hemoglobin may be due to differing iron levels in the chicken meal vs. the cricket meal, which was not measured in this study. However, each diet had the same inclusion level of the mineral premix, containing iron. The treatment differences among BUN and hemoglobin are not of clinical concern due to blood parameters remaining within the desired reference intervals for healthy dogs. Blood values outside desired reference intervals did occur based on individual dogs but were minimal. Overall, blood parameters were consistent throughout treatments indicating no impact on health status with dietary treatment.

### Conclusion

The study described the effect of graded levels of cricket meal in diets fed to adult dogs. Inclusion of cricket meal in canine diets can serve as an acceptable source of protein when compared with a control diet with chicken meal as a protein source. The maintenance of acceptable fecal characteristics and blood parameters throughout the duration of the study indicates that there were no adverse health effects while animals were fed dietary treatment. Differences in apparent digestibility, likely resulting from the increase in fiber, may drive decision on optimal inclusion level of cricket meal fed to adult dogs. Future research is needed to investigate the potential functionality of the chitin component in cricket meal. It would also be beneficial to investigate the health status of dogs resulting from longer-term feeding of diets containing cricket meal.

### Acknowledgment

This work was partially funded by Jiminy's LLC and all lab analysis was performed independently at Iowa State University.

### Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

### Literature Cited

- Barker, D., M. P. Fitzpatrick, and E. S. Dierenfeld. 1998. Nutrient composition of selected whole invertebrates. *Zoo Biol.* 17:123–134. doi:10.1002/(SICI)1098-2361(1998)17:2
- Bosch, G., J. J. M. Vervoort, and W. H. Hendriks. 2016. In vitro digestibility and fermentability of selected insects for dog foods. *Anim. Feed Sci. Tech.* 221:174–184. doi:10.1016/j.anifeedsci.2016.08.018
- 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
- Bueno, L., F. Praddaude, J. Fioramonti, and Y. Ruckebusch. 1981. Effect of dietary fiber on gastrointestinal motility and jejunal transit time in dogs. *Gastroenterology* 80:701–707. doi:10.1016/0016-5085(81)90129-3
- Castrillo, C., F. Vicente, and J. A. Guada. 2001. The effect of crude fibre on apparent digestibility and digestible energy content of extruded dog foods. *J. Anim. Physiol. Anim. Nutr. (Berl)*. 85:231–236. doi:10.1046/j.1439-0396.2001.00329.x
- Cole, J. T., G. C. Fahey Jr, N. R. Merchen, A. R. Patil, S. M. Murray, H. S. Hussein, and J. L. Brent Jr. 1999. Soybean hulls as a dietary fiber source for dogs. *J. Anim. Sci.* 77:917–924. doi:10.2527/1999.774917x
- DeFoliart, G. R., M. D. Finke, and M. L. Sunde. 1982. Potential value of the Mormon cricket (Orthoptera: Tettigoniidae) harvested as a high-protein feed for poultry. *J. Econ. Entomol.* 75:848–852. doi:10.1093/jee/75.5.848
- Diez, M., J. L. Hornick, P. Baldwin, C. Van Eenaeme, and L. Istasse. 1998. The influence of sugar-beet fibre, guar gum and inulin on nutrient digestibility, water consumption and plasma metabolites in healthy Beagle dogs. *Res. Vet. Sci.* 64:91–96. doi:10.1016/s0034-5288(98)90001-7
- Dumas, A., T. Raggi, J. Barkhouse, E. Lewis, and E. Weltzien. 2018. The oil fraction and partially defatted meal of black soldier fly larvae (*Hermetia illucens*) affect differently growth performance, feed efficiency, nutrient deposition, blood glucose, and lipid digestibility of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 492:24–34. doi:10.1016/j.aquaculture.2018.03.038
- Fahey, G. C. Jr, N. R. Merchen, J. E. Corbin, A. K. Hamilton, K. A. Serbe, S. M. Lewis, and D. A. Hirakawa. 1990. Dietary fiber for dogs: i. Effects of graded levels of dietary beet pulp on nutrient intake, digestibility, metabolizable energy and digesta mean retention time. *J. Anim. Sci.* 68:4221–4228. doi:10.2527/1990.68124221x
- Finke, M. D. 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. *Zoo Biol.* 21:269–285. doi:10.1002/zoo.10031
- Finke, M. D., G. R. DeFoliart, and N. J. Benevenga. 1987. Use of a four-parameter logistic model to evaluate the protein quality of mixtures of Mormon cricket meal and corn gluten meal in rats. *J. Nutr.* 117:1740–1750. doi:10.1093/jn/117.10.1740
- Finke, M. D., G. R. DeFoliart, and N. J. Benevenga. 1989. Use of a four-parameter logistic model to evaluate the quality of the protein from three insect species when fed to rats. *J. Nutr.* 119:864–871. doi:10.1093/jn/119.6.864
- Hosten, A. O. 1990. BUN and creatinine. In: Walker, H., W. Hall, and J. Hurst, editors. *Clinical methods: the history, physical, and laboratory examinations*. 3rd ed. Boston (MA): Butterworth Heinemann; p. 874–878.
- Jarett, J. K., A. Carlson, M. Rossoni Seroo, J. Strickland, L. Serfilippi, and H. H. Ganz. 2019. Diets with and without edible cricket support a similar level of diversity in the gut microbiome of dogs. *PeerJ.* 7:e7661. doi:10.7717/peerj.7661

- Koutsos, L., A. McComb, and M. Finke. 2019. Insect composition and uses in animal feeding applications: a brief review. *Ann. Entomol. Soc. Am.* 112:544–551. doi:10.1093/aesa/saz033
- Makkar, H. P., G. Tran, V. Heuzé, 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.anifeeds.2014.07.008
- McPherson-Kay, R. 1987. Fiber, stool bulk, and bile acid output: implications for colon cancer risk. *Prev. Med.* 16:540–544. doi:10.1016/0091-7435(87)90069-7
- Miech, P., J. E. Lindberg, A. Berggren, T. Chhay, and A. Jansson. 2017. Apparent faecal digestibility and nitrogen retention in piglets fed whole and peeled Cambodian field cricket meal. *J. Insects as Food Feed.* 3:279–287. doi:10.3920/JIFF2017.0019
- Moreki, J. C., B. Tiroesele, and S. C. Chiripasi. 2012. Prospects of utilizing insects as alternative sources of protein in poultry diets in Botswana: a review. *J. Anim. Sci. Adv.* 2:649–658.
- Nakagaki, B. J., M. L. Sunde, and G. R. DeFoliart. 1987. Protein quality of the house cricket, *Acheta domesticus*, when fed to broiler chicks. *Poult. Sci.* 66:1367–1371. doi:10.3382/ps.0661367
- National Research Council (NRC). 2006. *Nutrient requirements of dogs and cats*. Washington (DC): National Academies Press.
- National Research Council (NRC). 2012. *Nutrient requirements of swine. Committee on nutrient requirements of swine. 11th rev. ed.* Washington (DC): National Academies Press.
- Ngoan, L. D., L. V. An, B. Ogle, and J. E. Lindberg. 2000. Ensiling techniques for shrimp by-products and their nutritive value for pigs. *Asian-Austral. J. Anim. Sci.* 13:1278–1284. doi:10.5713/ajas.2000.1278
- Ngoan, L. D. and J. E. Lindberg. 2001. Ileal and total tract digestibility in growing pigs fed cassava root meal and rice bran diets with inclusion of fish meal and fresh or ensiled shrimp by-products. *Asian-Austral. J. Anim. Sci.* 14:216–223. doi:10.5713/ajas.2001.216
- Oonincx, D. G. A., and I. J. M. de Boer. 2012. Environmental impact of the production of mealworms as a protein source for humans – a life cycle assessment. *PLoS ONE* 7:1–5. doi:10.1371/journal.pone.0051145
- Poelaert, C., F. Francis, T. Alabi, R. Caparros Megido, B. Crahay, J. Bindelle, and Y. Beckers. 2018. Protein value of two insects, subjected to various heat treatments, using growing rats and the protein digestibility-corrected amino acid source. *J. Insects Food Feed.* 4:77–87. doi:10.3920/JIFF2017.0003
- Salomone, R., G. Saija, G. Mondello, A. Giannetto, S. Fasulo, and D. Savastano. 2017. Environmental impact of food waste bioconversion by insects: application of Life Cycle Assessment to process using *Hermetia illucens*. *J. Clean. Prod.* 140:890–905. doi:10.1016/j.jclepro.2016.06.154
- Sunvold, G. D., G. C. Fahey Jr, N. R. Merchen, E. C. Titgemeyer, L. D. Bourquin, L. L. Bauer, and G. A. Reinhart. 1995. Dietary fiber for dogs: IV. In vitro fermentation of selected fiber sources by dog fecal inoculum and in vivo digestion and metabolism of fiber-supplemented diets. *J. Anim. Sci.* 73:1099–1109. doi:10.2527/1995.7341099x
- Van Huis, A., J. Van Itterbeek, H. Klunde, E. Mertens, A. Holloran, G. Muir, and P. Vantomme. 2013. *Edible insects: future prospects for food and feed security*. FAO Forestry Paper 171. Rome (Italy): Food and Agriculture Organization of the United Nations (FAO); p.187.
- Wang, D., S. W. Zhai, C. X. Zhang, Y. Y. Bai, S. H. An, and Y. N. Xu. 2005. Evaluation on Nutritional Value of Field Crickets as a Poultry Feedstuff. *Asian-Australas. J. Anim. Sci.* 18:667–670. doi:10.5713/ajas.2005.667