Companion Animal Nutrition

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

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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 (Gryllodes 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 (Oonincx 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.
as they provide information about nutritional adequacy beyond evaluation of novel foods, it is important to have such studies. Miech et al. (2017) reported that whole crickets was 48% while that of fishmeal was 31% (Miech 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, 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 pigs. 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 ± 2.5 yr old with an initial body weight of 9.69 ± 1.9 kg (mean ± SD), were enrolled in this study. All animals were healthy, passing a veterinary physical examination and baseline hematoloy 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 ft² 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 (Gryllodes 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 µm). 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 analyses. 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 hematoloy and chemistry profiles. The sample was split into two collections 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

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| 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 |
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 1. Ingredient composition of diets

| Ingredient | 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    |
| Choline chloride 60% | 0.10   | 0.10    | 0.10    | 0.10    |
| Fish oil   | 0.25    | 0.25    | 0.25    | 0.25    |
| LANI vitamin premix1 | 0.10   | 0.10    | 0.10    | 0.10    |
| LANI trace mineral premix2 | 0.05   | 0.05    | 0.05    | 0.05    |
| LANI organic trace mineral premix1 | 0.01   | 0.01    | 0.01    | 0.01    |
| LANI Naturox Plus4 | 0.04   | 0.04    | 0.04    | 0.04    |

1LANI 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).
2LANI trace mineral premix (calcium carbonate, zinc sulfate, ferrous sulfate, copper sulfate, mineral oil, manganous oxide, sodium selenite, and calcium iodate).
3LANI 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 dihydriodide).
4LANI Naturox Plus (amorphous silicon dioxide, citric acid, natural mixed tocopherols, vegetable oil, and rosemary extract).

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          | ND1  |
| 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 recovery2 | 87.19 |

1Not determined.
2Amino 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 4,932 kcal/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

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

| Item          | 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                     | 0%     | 8%     | 16%    | 24%    | SEM   | Treatment Linear Quadratic Cubic |
|--------------------------|--------|--------|--------|--------|-------|-------------------------------|
| Intake                   |        |        |        |        |       | \( P = 0.385 \) \( 0.944 \) \( 0.322 \) \( 0.155 \) |
| Feed intake, g AF/d      | 231    | 193    | 227    | 222    | 16.81 | 0.385                         |
| Feed intake, g DM/d      | 213    | 178    | 211    | 208    | 15.61 | 0.380                         |
| GE intake, kcal/d        | 1,043  | 873    | 1,039  | 1,023  | 76.78 | 0.354                         |
| Output                   |        |        |        |        |       |                               |
| Fecal output, g as-is/d  | 64.8*  | 66.2*  | 70.3*  | 93.4*  | 7.16  | 0.030                         |
| Fecal output, g DM/d     | 23.4*  | 24.0*  | 26.4*  | 33.6*  | 2.44  | 0.024                         |
| Fecal score              | 3.40   | 3.44   | 3.47   | 3.43   | 0.03  | 0.336                         |
| Fecal pH                 | 6.53   | 6.36   | 6.19   | 6.18   | 0.14  | 0.232                         |
| Apparent digestibility   |        |        |        |        |       |                               |
| DM, %                    | 88.9*  | 86.5*  | 87.3*  | 83.9*  | 0.68  | <0.001                        |
| OM, %                    | 91.5*  | 89.4*  | 90.0*  | 86.8*  | 0.54  | <0.001                        |
| Crude Protein, %         | 88.2*  | 84.9*  | 86.0*  | 82.1*  | 0.74  | <0.001                        |
| Fat, %                   | 96.4*  | 95.7*  | 96.0*  | 94.8*  | 0.22  | <0.001                        |
| TDF, %                   | 57.5*  | 43.7*  | 61.3*  | 46.3*  | 2.81  | <0.001                        |
| GE, %                    | 92.4*  | 90.4*  | 90.8*  | 88.3*  | 0.49  | <0.001                        |

*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                             | 0%    | 8%    | 16%   | 24%   | SEM | Treatment | Linear | Quadratic | Cubic | Reference interval1 |
|----------------------------------|-------|-------|-------|-------|-----|-----------|--------|-----------|-------|---------------------|
| BUN, mg/dL                       | 11.7a | 11.3b | 13.2a | 12.2a | 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     | 0.204  | 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      | 10.5  | 10.1  | 14.2  | 12.3  | 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          |

1Reference intervals are laboratory specific.

a–cMeans 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                        | 0%           | 8%           | 16%          | 24%          | SEM          | Treatment     | Linear   | Quadratic | Cubic     | P-value |
|-----------------------------|--------------|--------------|--------------|--------------|--------------|---------------|----------|-----------|-----------|---------|
| White blood cell count (WBC), 10⁶/mm³ | 7.64         | 7.36         | 7.76         | 7.68         | 0.57         | 0.958         | 0.841    | 0.841     | 0.863     | 0.637   |
| Red blood cell count (RBC), 10⁶/mm³ | 6.83         | 6.77         | 6.61         | 6.51         | 0.82         | 0.197         | 0.036    | 0.036     | 0.096     | 0.757   |
| Hemoglobin, g/dL             | 15.7          | 15.5,a,b     | 15.1,b,c     | 14.9c        | 0.22         | 0.044         | 0.009    | 0.009     | 0.009     | 0.769   |
| Hematocrit, %                | 51.1          | 49.6         | 48.4         | 48.0         | 0.81         | 0.22          | 0.096    | 0.096     | 0.096     | 0.540   |
| Mean corpuscular volume, um³ | 74.4          | 73.6         | 73.1         | 74.0         | 1.05         | 0.73          | 0.533    | 0.533     | 0.533     | 0.693   |
| Mean corpuscular hemoglobin, ug/dL | 23.0          | 23.0         | 22.8         | 23.0         | 0.62         | 0.044         | 0.009    | 0.009     | 0.009     | 0.901   |
| Absolute platelets, 10⁶/mm³   | 272           | 264          | 317          | 285          | 14.42        | 0.179         | 0.078    | 0.078     | 0.078     | 0.769   |
| Absolute monocytes, 10⁶/mm³   | 377           | 391          | 441          | 392          | 64.45        | 0.179         | 0.078    | 0.078     | 0.078     | 0.578   |
| Absolute eosinophils, 10⁶/mm³ | 306           | 351          | 394          | 360          | 53.39        | 0.179         | 0.078    | 0.078     | 0.078     | 0.578   |
| Absolute basophils, 10⁶/mm³   | 0.00          | 0.00         | 0.00         | 0.00         | 0.00         | 0.00          | 0.00     | 0.00      | 0.00      | 0.00    |
| Absolute bands, 10⁶/mm³       | 0.00          | 0.00         | 0.00         | 0.00         | 0.00         | 0.00          | 0.00     | 0.00      | 0.00      | 0.00    |
| Absolute lymphocytes, 10⁶/mm³ | 1,903         | 2,010        | 2,058        | 1,885        | 102.97       | 0.179         | 0.078    | 0.078     | 0.078     | 0.901   |

1Reference intervals are laboratory specific.

Note: Means within a row having a common superscript letter are different (P < 0.05).

## 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 (< 0.001). Linear (P < 0.001) and cubic (P < 0.05) relationships were observed in DM, fat, 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., 2018).
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 digestibly 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 (Poelert et al., 2018).

**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

Buono, 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. Cuada. 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.1079-9424.2001.00329.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:10.2527/1999.7774917x

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.

Jaret, J. K., A. Carlson, M. Rossini Serao, 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

**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.

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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/asesa/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.anifeedsci.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
Oomi, 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