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Digestibility and safety of dry black soldier fly larvae meal and black soldier fly larvae oil in dogs

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Abstract

Two trials were conducted to assess the acceptance, safety and digestibility of diets containing various inclusion levels of partially defatted black soldier fly (*Hermetia illucens*) larvae (**BSFL**) meal and BSFL oil by dogs. In trial 1, 5 extruded diets were evaluated for acceptance in adult Beagle dogs (n = 20; 10 male, 10 female) during a 48-hr period. Diets contained graded levels of BSFL meal (5.0%, 10.0%, and 20.0%), or graded levels of BSFL oil (2.5% and 5.0%), and all diets were well accepted. Thus, a digestibility trial (trial 2) was run with 56 adult dogs (16 male, 40 female) allocated into 7 dietary treatments; dogs were offered an extruded control diet containing no BSFL meal or oil, or extruded diets where BSFL meal partially replaced poultry by-product meal and corn meal at dietary levels of 5%, 10%, or 20% inclusion, or diets with BSFL oil partially replacing poultry fat at a 1:1 ratio at levels of 1%, 2.5%, or 5% inclusion. The treatment diets were fed for 28 d, during which time dogs were monitored for health (via physical examinations, clinical observations, and blood chemistry and hematology) and ingredient evaluation (via body weight, feed consumption, stool observation, and fecal nutrient apparent total tract digestibility). There were no significant differences in body weight or food consumption between treatment groups ($P > 0.05$) and daily observations indicated that the general health of the animals was maintained throughout the study. Stool quality was maintained at 3.2 to 3.4 (on a 5-point scale with a score of 1 being watery diarrhea and a score of 5 being hard, dry, and crumbly) per treatment group over the fecal observation period (days 22 to 27), indicating a well formed, sticky stool. All group mean hematology and blood chemistry parameters remained within normal limits for dogs. Apparent total tract digestibility of dry matter, protein, fat, and calories was not affected by treatment ($P > 0.05$). In general, amino acid digestibility was not impacted by treatment although some minor changes were observed. Apparent total tract digestibility was high for all nutrients examined. Overall, it was concluded that BSFL meal and BSFL oil are well tolerated by dogs and their consumption results in no impact to physiology that would be concerning. Based on these data, BSFL meal and oil did not affect general health and could be included safely in dog diets.

Key words: alternative protein, black soldier fly larvae, digestibility, dogs, insects, pet food

Introduction

There is growing interest in the use of insect-derived ingredients for animal feed applications due to the potential for proportionally larger yields of protein and energy per unit area, the ability to utilize by-products of the food and feed industry as inputs, and supplement traditional protein sources to meet the needs of a growing population (Koutsos et al., 2019). In pet food, applications have been considered for dogs and cats for the aforementioned reasons (Bosch et al., 2014; McCusker et al., 2014), as a novel source of protein for pets with allergies,

Abbreviations

AOAC	Association of Official Analytical Chemists
BSFL	black soldier fly larvae

and for potential skin and gut health optimization. However, few publications have empirically evaluated the potential for application of insects in pet food. Whole dried insects, including black soldier fly (*Hermetia illucens*) larvae (**BSFL**), mealworm (*Tenebrio molitor*), Turkestan cockroach (*Shelfordella lateralis*), and tropical house crickets (*Gryllodes sigillatus*), were selected in olfactory tests at a similar rate as compared to commercial dog food (Kierończyk et al., 2018). In vitro digestibility in a simulated canine gastric and small intestinal digestion systems predicts excellent digestibility of nitrogen and amino acids (>90% and >87%, respectively) from BSFL, and predicts some microbial fermentation of chitin based upon generation of acetate, butyrate, and propionate (Bosch et al., 2016). Similarly, digestibility of BSFL of various ages was evaluated using the precision-fed cecetomized rooster assay and demonstrated that nutrient digestibility was high and that BSFL are a high-quality protein and amino acid source (Do et al., 2020). In vivo, adult Beagle dogs offered 3 different levels of cricket (*G. sigillatus*) meal (8%, 16%, or 24%) and a control (0% cricket meal) had similar feed intake and all blood parameters remained within acceptable reference intervals; though digestibility decreased with increasing cricket meal inclusion likely due to chitin content (Kilburn et al., 2020). Additionally, adult female Beagles fed diets containing up to 2% defatted BSFL meal for 42 d maintained normal blood parameters and had increased dry matter and crude protein digestibility compared to control diets (Lei et al., 2019). Most recently, BSFL meal was fed at 20% of an extruded diet to Beagle dogs for 5 wk and apparent total tract dry matter digestibility was slightly greater in BSFL meal fed dogs (83% vs. 82%) while apparent total tract protein digestibility was slightly lower (77% vs. 79%; Kröger et al., 2020). There were no major differences in immune parameters, or fecal quality when compared to a lamb-based extruded diet.

While BSFL-derived ingredients have not been well studied in companion animals, there is considerable research demonstrating efficacy and safety in poultry, swine, and aquaculture species. In poultry diets, BSFL can replace part or all of protein sources such as soybean meal or fish meal (Oluokun, 2000; Makkar et al., 2014; Cullere et al., 2016; Maurer et al., 2016; Abd El-Hack et al., 2020). In swine diets, research dates back to 1977 (Newton et al., 1977) and there has been particular interest in using BSFL to feed during the nursery phase. Black soldier fly larvae meal can replace soybean meal and grease in nursery pig diets (Driemeyer, 2016), and BSFL oil has been demonstrated to improve growth performance (van Heugten et al., 2019). In aquaculture, BSFL meal can be used as a partial replacement for fishmeal in the diets of many fish species (Sánchez-Muros et al., 2014; Henry et al., 2015).

Overall, BSFL-derived ingredients appear to be a safe and effective alternative to other high-quality protein and energy sources in animal feeds. Thus, BSFL has the potential to be formulated into nutritionally balanced complete feed for companion animals. The purpose of this trial was to evaluate the acceptance, and then general performance (feed intake and body weight maintenance), nutrient apparent total tract digestibility and blood parameters indicative of health status in healthy adult dogs fed BSFL meal and BSFL oil.

Materials and Methods

Study design

Both trial 1 and trial 2 were conducted in a USDA kennel (registration number 23-R-0126). All research protocols were reviewed and approved by the Summit Ridge Farms' Institutional Animal Care and Use Committee and were in compliance with the Animal Welfare Act. Dogs were individually housed in indoor enclosures of 15.5 square feet of floor space, with standard light cycles, temperature and sanitation per Animal Welfare Act guidelines. Fresh tap water, fit for human consumption, was available ad libitum by means of an automatic watering system.

Trial 1: acceptance of BSFL meal and oil

The purpose of trial 1 was to assess the acceptance of BSFL meal and oil by dogs. The general nutrient composition of BSFL oil and meal is presented in Tables 1 and 2.

Forty individually housed, purpose-bred, Beagle dogs were used to evaluate the diets. In each treatment, 20 Beagle dogs (10 male, 10 female) were offered 1 of the 5 different test diets containing BSFL-derived ingredients for 2 consecutive days. Graded levels of BSFL meal (5.0%, 10.0%, and 20.0%) or BSFL oil (2.5% and 5.0%; external application) were evaluated for acceptance; ingredient inclusion rates and analyzed nutrient content are provided in Tables 3 and 4, respectively). Groupings of 20 dogs tested the diet treatments in the following order: Group A: BSFL meal 5%, BSFL meal 10%, and BSFL oil 5%; Group B: BSFL meal 20%, BSFL oil 2.5%. The diets were extruded at Extru-Tech, Inc. (Sabetha, KS). Dogs were offered 400 g of test diet per day (which exceeded the predicted daily energy requirement of Beagles) for a 2-d period, and bowls were presented for 30 min once daily. The dependent variable for this trial was intake, measured as grams of consumption per day and modified Atwater -metabolizable energy was calculated

Table 1. General nutrient composition[1] of black soldier fly (*Hermetia illucens*) larvae (BSFL) meal on an as-fed basis

Nutrient	BSFL meal
Moisture, %	3.85
Crude protein, %	46.0
Crude fat, %	14.7
Acid detergent fiber, %	10.2
Ash, %	8.90
Calcium, %	2.14
Phosphorus, %	1.03
Arginine, %	2.00
Glycine + serine, %	3.65
Histidine, %	1.14
Isoleucine, %	1.69
Leucine, %	2.89
Lysine, %	2.19
Methionine, %	0.63
Methionine + cystine, %	1.03
Phenylalanine, %	1.68
Phenylalanine + tyrosine, %	4.10
Threonine, %	1.20
Tryptophan, %	0.50
Valine, %	2.30
Lauric acid, %	5.11
Linoleic acid, %	2.56
Linolenic acid, %	0.22

[1]EnviroFlight, LLC internal data.

as: [(3.5 × crude protein) + (8.5 × crude fat) + (3.5 × nitrogen-free extract)] × 10, where nitrogen-free extract = 100 – (crude protein + crude fat + crude fiber + moisture + ash). Data were analyzed by 1-way ANOVA (SigmaPlot Software, San Jose, CA) for effect of diet treatment on intake and means comparisons were assessed using Tukey's test. Significance was set at $P < 0.05$.

Trial 2: performance, digestibility, and blood parameters in response to BSFL meal and oil. Individually housed, apparently healthy, purpose-bred adult Beagle dogs (n = 56 dogs; 16 males, 40 females; age = 3.8 ± 1.57 yr old (mean ± SD), age range 1 to 8 yr old; body weight = 9.5 ± 1.68 kg (mean ± SD); mean body condition score = 3 of 5 for all animals) were used in trial 2. Before study initiation, dogs were fed a standard adult canine maintenance diet, were meal fed, and diet levels offered were based on maintenance of body weight. Trial 2 was conducted as a completely randomized design with dogs distributed into 7 groups with blocking for age, sex, and initial body weight. Dogs were assigned to 1 of 7 diets: control (Diet 1), diets containing graded levels of BSFL meal (5.0%, 10.0%, and 20.0%; diets 2 to 4, respectively), or diets containing graded levels of BSFL oil (1.0%, 2.5%, and 5.0%; diets 5 to 7, respectively). All diets were based on standard feed ingredients and met or exceeded the Association of American Feed Control Officials recommendations for dogs. Ingredient inclusion rates and nutrient analyses of diets are presented in Tables 3 and 5, respectively. Diets were formulated around macronutrients with fixed vitamin and mineral premix inclusion; thus, micronutrients varied slightly between diets.

Dogs were fed their assigned diets for 28 d, during which time, the assigned diets were the sole source of food. Personnel were not blinded to the diets offered. Diets were offered once daily, and feed amounts were adjusted weekly or more often as needed, to maintain each animal's body weight. Individual body weights were measured and recorded weekly throughout the study. Food consumption was recorded daily. Animals were observed daily and any adverse reactions were noted. Specifically, a veterinarian performed a complete physical examination for general health and body condition on all dogs at study initiation. Qualified personnel performed clinical observations twice daily in accordance with Summit Ridge Farms' Program of Veterinary Care and SOP VC-003. No medications or vaccines were administered during the course of the study. All animals remained in apparent good health during the study and none were removed from the study.

Blood was collected at onset and the completion of the trial. Briefly, 5 mL of blood was collected per dog via jugular venipuncture into sterile syringes. Samples were split into red-top serum separator tubes and lavender-top ethylenediaminetetraacetic acid tubes. Red-top tubes were spun in a refrigerated centrifuge for 15 min at 3,000 rpm after being allowed to clot. Lavender-top tubes were placed on a rocker and allowed to adequately mix with the anticoagulant. Blood samples were then shipped on ice for analysis (Antech Diagnostics, Memphis, TN). A white blood cell count, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, and platelet count along with a complete differential was conducted. The serum chemistry analysis consisted of the following 22 parameters: glucose, urea nitrogen, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, cholesterol, calcium, phosphorus, sodium, potassium, chloride, albumin-globulin ratio, blood urea nitrogen/creatinine ratio, globulin, magnesium, γ-glutamyl transpeptidase, triglycerides, and creatine phosphokinase. For each dog the change in blood parameter over the course of the trial was calculated.

Fecal collection and fecal scoring were conducted on days 22 to 27 of the trial. Qualified personnel (not blinded to treatment) performed fresh stool quality observations at least 3 times daily

Table 2. General nutrient composition[1] of black soldier fly (*Hermetia illucens*) larvae (BSFL) oil on an as-fed basis

Nutrient	BSFL oil
Moisture, %	0.25
Crudefat, %	97.3
Lauric acid, %	38.4
Linoleic acid, %	17.5
Linolenic acid, %	1.33
Arachidonic acid, %	0.01

[1]EnviroFlight, LLC internal data.

Table 3. Ingredient inclusion rates (%) for diet treatments for an acceptance trial (trial 1) and a digestibility trial (trial 2), including control diet (0.0% BSFL[1]) and 3 treatment levels of BSFL meal (5.0%, 10.0%, and 20.0%) and BSFL oil (1.0%, 2.5%, 5.0%)

Ingredient name	Control	5% BSFL meal	10% BSFL meal	20% BSFL meal	1% BSFL oil	2.5% BSFL oil	5% BSFL oil
Poultry by-product meal	39.96	39.96	36.00	26.00	39.96	39.96	39.96
Brewers rice	35.00	35.00	35.00	35.00	35.00	35.00	35.00
BSFL meal	0.00	5.00	10.00	20.00	0.00	0.00	0.00
Corn	12.00	7.04	6.00	6.00	12.00	12.00	12.00
Beet pulp	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Chicken fat	5.00	5.00	5.00	5.00	4.00	2.50	0.00
BSFL oil	0.00	0.00	0.00	0.00	1.00	2.50	5.00
Dicalcium phosphate	1.34	1.30	1.30	1.30	1.34	1.34	1.34
Potassium chloride	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Salt (NaCl)	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Mineral premix	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10	0.10

[1]BSFL, black soldier fly larvae, *Hermetia illucens*.
Briefly, adult Beagle dogs (n = 20/dietary treatment for trial 1 and n = 8/dietary treatment for trial 2) were fed assigned diets for 2 d (trial 1) or for 28 d (trial 2).

Table 4. Analyzed nutrient content of diets from an acceptance trial (trial 1) on an as-fed basis

Nutrient	5.0% BSFL[1] meal	10.0% BSFL meal	20.0% BSFL meal	2.5% BSFL oil	5.0% BSFL oil
Moisture, %	7.85	7.36	6.92	7.74	8.33
Crude protein, %	32.1	34.7	35.7	33.1	32.6
Acid hydrolyzed fat, %	12.5	14.5	14.9	13.5	13.9
Ash, %	8.05	8.13	8.66	7.39	7.34
Crude fiber, %	2.25	2.76	3.60	1.76	1.60

[1]BSFL, black soldier fly larvae, *Hermetia illucens*.
Adult Beagle dogs (n = 20/dietary treatment) were offered assigned diets for 2 d, and intake was monitored daily.

Table 5. Analyzed nutrient content of diets from a digestibility trial (trial 2) on an as-fed basis

Nutrient	0.0% BSFL[1]	5.0% BSFL meal	10.0% BSFL meal	20.0% BSFL meal	1.0% BSFL oil	2.5% BSFL oil	5.0% BSFL oil
Moisture, %	5.90	5.41	5.92	6.52	5.98	6.27	6.42
Crude protein, %	31.3	38.2	34.2	31.7	32.8	28.9	30.5
Acid hydrolyzed fat, %	11.9	13.7	12.3	12.4	12.2	11.4	11.5
Ash, %	7.82	8.11	7.91	7.12	8.44	8.10	8.06
Crude fiber, %	0.47	0.30	1.11	1.65	0.80	0.54	0.66
Alanine, %	1.87	2.28	2.05	2.15	2.05	2.00	1.81
Arginine, %	1.93	2.36	1.99	1.99	2.08	2.05	1.83
Aspartic acid, %	2.39	2.89	2.55	2.73	2.55	2.50	2.30
Glutamic acid, %	4.00	4.77	4.18	4.48	4.29	4.32	3.90
Glycine, %	2.43	2.92	2.62	2.36	2.61	2.45	2.29
Histidine, %	0.59	0.72	0.67	0.71	0.64	0.59	0.59
Isoleucine, %	1.08	1.34	1.14	1.27	1.15	1.12	1.03
Leucine, %	2.04	2.40	2.07	2.21	2.12	2.15	1.88
Lysine, %	1.61	1.98	1.65	1.60	1.72	1.72	1.56
Methionine, %	0.58	0.77	0.62	0.58	0.67	0.61	0.64
Methionine + cystine, %	1.00	1.21	1.01	1.14	1.13	0.95	1.01
Phenylalanine, %	1.12	1.36	1.18	1.27	1.22	1.20	1.07
Phenylalanine + tyrosine, %	2.07	2.50	2.29	2.55	2.22	2.19	2.06
Proline, %	1.76	2.17	1.88	2.00	1.91	1.89	1.71
Serine, %	1.19	1.41	1.26	1.35	1.30	1.28	1.15
Threonine, %	0.79	0.98	0.82	0.90	0.85	0.86	0.80
Tryptophan, %	0.33	0.34	0.37	0.42	0.37	0.35	0.33
Valine, %	1.39	1.70	1.47	1.66	1.44	1.44	1.31
ME[2], kcal/kg	3,600	3,700	3,600	3,600	3,600	3,500	3,500
GE[3], kcal/kg	4,600	4,700	4,700	4,700	4,600	4,500	4,000

[1]BSFL, black soldier fly larvae, *Hermetia illucens*.
[2]Metabolizable energy (ME) Atwater calculated.
[3]Gross energy (GE) obtained from bomb calorimetry.
Adult Beagle dogs (n = 8/dietary treatment) were fed assigned diets for 28 d, and intake, body weight, stool quality and blood parameters were assessed.

during the fecal collection periods. The following scale was used during fecal consistency observations: 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, and crumbly. Fecal samples were weighed, and then frozen before analysis for digestibility.

Fecal samples and diet samples were analyzed by a commercial lab (Midwest Laboratories, Omaha NE), according to approved Association of Official Analytical Chemists (**AOAC**) analytical methodology for the following: moisture, fat, protein, ash, fiber, and calories (respectively: AOAC 930.15, 954.02 (mod), 990.03, 942.05, American Oil Chemists' Society Ba 6a-05, ASTM D 5865-13). Calculations for the apparent total tract digestibility of dry matter, protein, fat and calories were performed. One composite per dog of fecal material collected on days 22 to 27 was analyzed for amino acids using the following methodology: aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, total lysine, histidine, arginine [AOAC 994.12 (Alt. III)], cystine, methionine [AOAC 994.12 (Alt. I)], tryptophan [AOAC 994.12 (mod)], and the apparent total tract digestibility for amino acids was calculated.

Diet apparent total tract digestibility was determined based on analyses of diets and feces, using the following equation where "*Nutrient*" was dry matter, protein, fat, or gross energy per gram:

$$\begin{aligned}&\text{Nutrient apparent total tract digestibility}\\&\quad=[\{(\text{total food consumed})\times(\%\,\text{nutrient of food})\}\\&\qquad-\{(\text{total weight of stool})\times(\%\,\text{nutrient of stool})\}]\\&\qquad\div\{(\text{total food consumed})\times(\%\,\text{nutrient of food})\}\end{aligned}$$

$$\text{gross energy (kcal/g)} = \{(5.65 \times \text{crude protein}) + (4.15 \times \text{nitrogen} - \text{free extract}) + (9.4 \times \text{crude fat})\} \div 100$$

Statistical analysis

The experimental unit was the individual dog. Data were examined for normality and homogeneity of variance (SigmaPlot, San Jose, CA). If either were not achieved ($P < 0.05$), ANOVA on RANKS was run to examine differences due to treatment. If both were achieved, 1-way ANOVA was run to examine differences due to treatment. If main effect of treatment was significant ($P < 0.05$), Tukey's test was used to determine differences between means. The number of animals used was sufficient to achieve statistical power. No animals were removed from the trial.

Results

Trial 1

Absolute intake and metabolizable energy intake are presented in Table 6. Diet did not have a significant effect on intake or metabolizable energy intake.

Trial 2

Body weight, stool quality, and diet consumption

The summary of the group mean weekly body weights and daily diet consumption is presented in Table 7. Fecal scores were maintained at an average score of 3.3 ± 0.04 (mean ± SEM) with a range of 3.2 to 3.4. There were no significant changes in body weight, fecal scores, or diet consumption over time due to diet treatment.

Blood analysis

Initial, final, and the change between blood parameters over time for serum chemistry and hematology results are presented in Tables 8 and 9, respectively. All hematology and serum chemistry values remained within normal reference ranges (Fielder, 2015) throughout the trial. There was a main effect of dietary treatment on the change in alanine aminotransferase over the course of the trial; however no meaningful response to dietary inclusion of meal or oil was noted, and thus this effect is likely due to random variation.

Digestibility

The apparent total tract digestibility of dry matter, protein, fat, and gross energy was not significantly different due to dietary treatment (Table 10). The apparent total tract digestibility of amino acids was significantly different due to dietary treatment for cystine, tyrosine, and tryptophan, but did not differ for other amino acids. Dogs fed 20% BSFL meal had higher cystine digestibility than those fed 5% BSFL meal or 1% BSFL oil but were not different than those fed control, and likely does not reflect biologically relevant differences. Although there was a main effect of diet on digestibility for tyrosine ($P = 0.03$) there were no statistically significant differences between treatment means. Tryptophan digestibility increased with increasing BSFL meal inclusion, and dogs fed 20% BSFL meal had greater digestibility than those fed 0% BSFL meal. Numerically, dogs fed BSFL oil had higher digestibility for tryptophan as compared to those fed Control diet, though this was only statistically significant for those fed 1.0 and 2.5% BSFL oil.

Discussion

Dietary inclusion of up to 20% BSFL meal and 5% BSFL oil were readily accepted by adult dogs (trial 1). Higher levels of these

Table 6. Absolute daily intake and calculated metabolizable energy (ME) intake of dogs fed BSFL[1] for an acceptance trial (trial 1)

Intake measures	5.0% BSFL meal	10.0% BSFL meal	20.0% BSFL meal	2.5% BSFL oil	5.0% BSFL oil
Absolute intake, g/d	237.3 ± 15.66	225.1 ± 13.05	210.2 ± 15.83	207.3 ± 15.48	249.3 ± 15.18
ME intake, kcal	827 ± 54.6	800 ± 46.4	746 ± 56.2	729 ± 54.5	882 ± 53.7
	(406 to 1394)	(489 to 1421)	(355 to 1315)	(331 to 1342)	(578 to 1414)

[1]BSFL, black soldier fly larvae, *Hermetia illucens*.
Adult Beagle dogs (n = 20/dietary treatment) were offered assigned diets for 2 d, and intake was monitored daily. Data are presented as mean ± SEM (Min – Max).

Table 7. Group means and pooled SEM of weekly body weights (BW) and daily feed consumption over study period of 28 d of dogs in a digestibility study (trial 2) fed diet treatments containing varying amounts of BSFL[1]

	0.0% BSFL	5.0% BSFL meal	10.0% BSFL meal	20.0% BSFL meal	1.0% BSFL oil	2.5% BSFL oil	5.0% BSFL oil	SEM
Initial BW, kg	9.3	9.6	9.5	9.4	9.5	9.5	9.5	0.59
Week 1 BW, kg	9.2	9.5	9.4	9.3	9.4	9.3	9.3	0.62
Week 2 BW, kg	9.2	9.5	9.3	9.2	9.3	9.3	9.3	0.64
Week 3 BW, kg	9.2	9.5	9.3	9.2	9.3	9.3	9.2	0.65
Week 4 BW, kg	9.1	9.5	9.3	9.2	9.3	9.4	9.2	0.65
BW change weeks 1 to 4, kg	−0.2	−0.1	−0.2	−0.2	−0.2	−0.1	−0.3	0.10
Daily consumption, g	184	202	192	182	204	208	195	10.8

[1]BSFL, black soldier fly larvae, *Hermetia illucens*.
Adult Beagle dogs (n = 8/dietary treatment) were fed assigned diets for 28 d, and intake, body weight, stool quality, and blood parameters were assessed.

Table 8. Serum chemistry results (group means ± pooled SEMs) from dogs fed various levels of BSFL-derived ingredients for a digestibility trial (trial 2) and main effect of treatment (P-values) for the change between blood parameters over time

Parameter	Initial	Final	P-value	Reference range[1]
Total protein, g/dL	6.3 ± 0.16	6.4 ± 0.16	0.616	5.0 to 7.4
Albumin, g/dL	3.6 ± 0.08	3.5 ± 0.09	0.946	2.7 to 4.4
Globulin, g/dL	2.7 ± 0.16	2.8 ± 0.16	0.581	1.6 to 3.6
A/G[2] ratio	1.4 ± 0.09	1.3 ± 0.09	0.693	0.8 to 2.0
AST, U/L	28 ± 2.0	31 ± 2.9	0.296	15 to 66
ALT, U/L	37 ± 4.1	47 ± 3.6	0.012	12 to 118
ALP, U/L	42 ± 7.8	42 ± 7.5	0.348	5 to 131
GGT, U/L	6 ± 0.7	5 ± 0.5	0.390	1 to 12
Bilirubin, mg/dL	0.2 ± 0.02	0.2 ± 0.02	0.545	0.1 to 0.3
Urea nitrogen, mg/dL	12 ± 0.7	13 ± 0.8	0.169	6 to 31
Creatinine, mg/dL	0.6 ± 0.03	0.6 ± 0.04	0.066	0.5 to 1.6
BUN/Creatinine	20 ± 0.4	23 ± 0.6	0.056	4 to 27
Phosphorus, mg/dL	3.7 ± 0.07	3.6 ± 0.08	0.267	2.5 to 6.0
Glucose, mg/dL	94 ± 2.7	87 ± 2.8	0.368	70 to 138
Calcium, mg/dL	9.8 ± 0.14	10.0 ± 0.12	0.416	8.9 to 11.4
Magnesium, mEq/L	1.6 ± 0.04	1.6 ± 0.05	0.082	1.5 to 2.5
Sodium, mEq/L	149 ± 0.6	147 ± 0.6	0.790	139 to 154
Potassium, mEq/L	4.5 ± 0.05	4.3 ± 0.04	0.088	3.6 to 5.5
Chloride, mEq/L	114 ± 0.2	112 ± 0.2	0.069	102 to 120
Cholesterol, mg/dL	189 ± 14.1	191 ± 16.8	0.319	92 to 324
Triglycerides, mg/dL	47 ± 3.5	50 ± 3.8	0.262	29 to 291
CPK, U/L	137 ± 6.1	154 ± 11.8	0.340	59 to 895

[1]References ranges from Fielder (2015).
[2]A/G, albumin-globulin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, γ -glutamyl transpeptidase; BUN, blood urea nitrogen; CPK, creatine phosphokinase.
Adult Beagle dogs (*n* = 8/dietary treatment) were fed assigned diets for 28 d, and intake, body weight, stool quality, and blood parameters were assessed.

Table 9. Hematology profile results (group means ± pooled SEMs) from dogs fed various levels of BSFL-derived ingredients for a digestibility trial (trial 2) and main effect of treatment (P-values) for the change between blood parameters over time

Parameter	Initial	Final	P-value	Reference Range[1]
WBC, 10^3/mm^3	8.3 ± 0.68	7.8 ± 0.71	0.489	4.0 to 15.5
RBC, 10^6/mm^3	7.3 ± 0.21	7.3 ± 0.21	0.716	4.8 to 9.3
Hemoglobin, g/dL	17.2 ± 0.45	16.9 ± 0.49	0.248	12.1 to 20.3
Hematocrit, %	53 ± 1.5	54 ± 1.4	0.614	36 to 60
MCV, µm^3	73 ± 0.7	74 ± 0.9	0.517	58 to 79
MCH, µg	23.5 ± 0.29	23.2 ± 0.30	0.128	19 to 28
MCHC, g/dL	32 ± 0.4	32 ± 0.2	0.232	30 to 38
Platelets, 10^3/mm^3	331 ± 28.8	328 ± 33.2	0.602	170 to 400
Polymorphonuclear cells, #/mL	5,545 ± 566.3	5,067 ± 571.2	0.389	2,060 to 10,600
Band cells, #/mL	13 ± 13.4	0 ± 0.0	n/a[2]	0 to 300
Lymphocytes, #/mL	2,039 ± 159.5	2,044 ± 182.0	0.531	690 to 4,500
Monocytes, #/mL	338 ± 61.2	341 ± 52.6	0.235	0 to 840
Eosinophils, #/mL	307 ± 54.7	309 ± 60.6	0.599	0-1,200
Basophils, #/mL	0 ± 0.0	0 ± 0.0	n/a[2]	0 to 150

[1]References ranges from Fielder (2015).
[2]WBC, white blood cell; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.
[3]Statistics not run due to single value.
Adult Beagle dogs (*n* = 8/dietary treatment) were fed assigned diets for 28 d and intake, body weight, stool quality and blood parameters were assessed.

ingredients were not assessed due to the focus on commercially relevant diet formulation (i.e., higher inclusion levels would have necessitated the use of purified or semi-purified ingredients to maintain isocaloric and isonitrogenous diet formulation). As a result of the acceptance data from trial 1, a longer-term trial was conducted to examine digestibility and indicators of health in response to graded levels of BSFL meal and oil. Twenty-eight days was chosen as an appropriate study period based on marked changes in biochemical parameters appearing in dogs fed a protein-deficient diet at 4 wk, such as serum urea nitrogen dropping below reference ranges and indicating protein deficiency (Davenport et al., 1994).

Body weight and feed consumption were maintained over an extended feeding period in adult dogs fed up to 20% BSFL meal and up to 5% BSFL oil (trial 2). Stool quality was maintained in an ideal range in all treatment groups, and apparent total tract

Table 10. Group means and pooled SEM of apparent total tract digestibility results of dogs enrolled in a digestibility trial (trial 2) on diet treatments containing varying amounts of BSFL[1] products

Apparent total tract digestibility	0.0% BSFL	5.0% BSFL meal	10.0% BSFL meal	20.0% BSFL meal	1.0% BSFL oil	2.5% BSFL oil	5.0% BSFL oil	SEM
Dry matter, %	90.0	88.4	89.6	88.5	91.1	91.0	90.0	0.86
Protein, %	89.3	89.3	89.6	87.9	91.0	90.2	89.4	0.88
Fat, %	96.3	96.6	96.4	96.7	96.8	96.6	96.0	0.34
Gross energy[2], %	92.5	91.8	92.3	91.2	93.6	93.4	91.7	0.64
Arginine, %	94.9	95.1	95.8	95.7	96.0	96.2	95.0	0.14
Histidine, %	92.6	92.9	93.7	93.8	94.1	93.6	9.29	0.20
Isoleucine, %	89.3	89.9	90.0	91.0	91.3	91.5	89.4	0.29
Leucine, %	91.3	91.5	91.6	91.8	92.5	93.2	91.0	0.25
Lysine, %	90.3	90.1	90.4	90.1	91.9	92.4	90.4	0.28
Methionine, %	91.3	92.7	92.3	92.2	93.9	93.4	92.3	0.22
Cystine, %	85.8^{abc}	84.4^{c}	85.6^{abc}	89.8^{a}	89.5^{ab}	85.2^{abc}	84.6^{bc}	0.41
Phenylalanine, %	90.7	91.0	91.6	92.1	92.6	93.0	90.7	0.25
Tyrosine, %	91.3	92.4	93.3	93.8	93.7	93.8	92.8	0.21
Threonine, %	90.4	90.9	91.2	91.9	92.2	92.9	90.9	0.26
Tryptophan, %	89.3^{b}	89.4^{b}	91.7^{ab}	93.7^{a}	93.3^{a}	94.0^{a}	91.9^{ab}	0.24
Valine, %	89.3	89.9	90.2	91.0	91.2	91.8	89.4	0.29

[1]BSFL, black soldier fly larvae, *Hermetia illucens*.
[2]Values used to calculate these numbers were obtained using calculated gross energy.
[a,b]Means in the same row with different superscripts are significantly different ($P < 0.05$).
Adult Beagle dogs (n = 8/dietary treatment) were fed assigned diets for 28 d, and intake, body weight, stool quality, and blood parameters were assessed.

digestibility of dry matter, protein, fat, and energy were similar between treatments. Apparent total tract digestibility of protein, fat, and dry matter were maintained at high levels with all BSFL ingredient inclusion levels (88.9%, 96.5%, and 89.9%, respectively). These digestibility data are comparable to high-quality protein sources for dogs, and similar or higher than reported in vitro dry matter digestibility of whole BSFL for dogs (81.4%; Bosch et al., 2016; 89.7%; Bosch et al., 2014) and *in vivo* dry matter digestibility when dogs were fed 2% BSFL meal (75.2%; Lei et al., 2019), or 20% BSFL meal (83.2%; Kröger et al., 2020). In Lei et al., 2019, the very low inclusion level of BSFL meal makes it likely that diet digestibility was influenced by the other (major) ingredients in the diet, rather than BSFL. Similar to results of this trial and others with BSFL meal, cricket meal inclusion up to 24% of the diet maintained high overall digestibility for dogs (>80% apparent dry matter digestibility; Kilburn et al., 2020). The relatively high apparent total tract digestibility of protein and energy observed in the current trial and other dog trials is supported by data in other species including broiler chickens (DeMarco et al., 2015; Khan, 2018) and young pigs (Biasato et al., 2019).

Apparent total tract amino acid digestibility, like protein, was relatively high for all amino acids studied and did not vary dramatically with BSFL meal inclusion level, with the exception of tryptophan, for which apparent digestibility increased with increasing BSFL meal inclusion. These data are similar to that found using a cecectomized rooster assay, which is typically used in place of more invasive measures in companion animals (Do et al., 2020). In work by Do et al. (2020), amino acid digestibility was found to be >90% for all amino acids except for valine (85%) and cysteine (79%) when cecectomized roosters were fed BSFL at a similar larval life stage. This is very similar to our data, in which apparent digestibility was generally >90% for all amino acids, with apparent cystine digestibility slightly lower. While apparent total tract digestibility has obvious limitations due to contributions of endogenous losses and potential microbial contributions, the relative similarity of responses between multiple assay systems lends confidence to these data.

Blood parameters indicative of canine health were maintained in normal ranges for the extended 28 d feeding period in adult dogs fed up to 20% BSFL meal and up to 5% BSFL oil (trial 2). This is similar to previous observations with BSFL meal and oil to dogs (Lei et al., 2019), and in poultry (Dabbou et al., 2018; Schiavone et al., 2018).

Overall, these trials demonstrate that BSFL meal and oil are readily accepted by dogs. Further, apparent total tract digestibility of macro-nutrients and amino acids were maintained at very high levels, as were body weight, stool quality, and blood parameters indicative of health and nutritional status. Based on these data, and similar observations in other species, it can be concluded that defatted BSFL meal and BSFL oil are well tolerated by dogs under the study conditions.

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Conflict of Interest Statement

The authors declare no real or perceived conflicts of interest, although all authors are employed by EnviroFlight LLC, a producer of BSFL meal and oil.

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