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RESEARCH ARTICLE

Black soldier fly larvae meal in an extruded food: effects on nutritional quality and health parameters in healthy adult cats

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Abstract

We aimed to evaluate the effects of including black soldier fly larvae meal (BSFL) meal in a dry extruded food on nutritional quality and some health aspects in healthy adult cats. Two dry extruded foods with either poultry meal (control) or 37.5% BSFL meal were fed to eight (3.8-5.2 kg BW; 2.3-y.o.) cats in a cross-over design with two 28-day periods. Food acceptance was recorded throughout the study and samples were collected during the last 7 days of each period for assessment of apparent total tract nutrient digestibility, faecal consistency, fermentation products and microbiota, and blood biochemistry and haematology. Foods were well-accepted and faeces were well-formed with optimal consistency scores. Digestibility values for dry matter, organic matter, nitrogen and gross energy were considered high for the BSFL meal-based food but lower than for the control food ($P < 0.05$). Unexpectedly, inclusion of BSFL meal had a profound impact on the intestinal microbial activity and composition as illustrated by increased faecal short-chain fatty acids ($P < 0.05$) and biogenic amines concentrations ($P < 0.05$) and reduced bacterial diversity ($P < 0.05$) and shifts in multiple genera (e.g. more *Bifidobacterium*) in the faeces ($P < 0.05$). Minor changes in haematology and serum biochemistry parameters were found and deemed not clinically relevant. Overall, this study showed that a BSFL meal-based extruded dry food is readily accepted by healthy adult cats, yields optimal faecal consistency, had suitable nutrient digestibilities and can support their health when fed for 28 days with new leads for impact on feline gut health.

Keywords

digestibility – faecal fermentation profiles – faecal microbiota – haematology – serum biochemistry

1 Introduction

Insects like the black soldier fly larvae (*Hermetia illucens* (L.), Diptera: Stratiomyidae; BSFL) have been proposed as protein-rich feed ingredients to support the growing demand for meat (Rumpold and Schlüter, 2013; Van Huis *et al.*, 2013). The BSFL is used as a protein source in pet foods and as such have been studied for palatability, nutritional quality and health effects in both dogs and cats. In cats, the first short-term studies have indicated

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that BSFL-based dry extruded foods can, depending on insect meal inclusion level, have variable palatability (acceptance), good nutrient digestibility, and good faecal consistency (reviewed by Bosch and Swanson, 2021). Furthermore, based on serum biochemistry and haematology, health was supported by a BSFL meal-based wet food (10% inclusion) fed for 7 weeks to adult cats (Do *et al.*, 2022) and a BSFL meal-based semi-synthetic food (4% inclusion) fed for 3 weeks to adult cats (Pezzali and Shoveller, 2021). Impacts of a BSFL meal-based dry extruded food on health has not been studied in cats but in dogs an extruded food with 20% BSFL meal did not negatively impact health (Kröger *et al.*, 2020).

Dietary fibres can impact microbial activity, intestinal health and faecal consistency. It is therefore of interest to study the potential shifts in microbiota and fermentation characteristics after feeding insect-based foods containing chitin as a fibre source. Studies in dogs fed a dry extruded food with banded cricket meal (*Gryllodes sigillatus*) (Jarrett *et al.*, 2019) and in cats fed wet foods containing 4% insect meal (Madagascar hissing cockroach *Gromphadorhina portentosa*, speckled cockroach *Nauphoeta cinerea* or superworm *Zophobas morio* larvae) (Reilly *et al.*, 2021) indicate that including insects in foods can shift gut microbial populations. The latter study did not observe changes in faecal fermentation products, which is in line with the low *in vitro* fermentability of indigestible insect parts by cat faecal microbiota observed by Bosch and Post (2019). Low fermentability of indigestible parts of insects was also found in dogs fed BSFL meal-based extruded food (20%) for 35 days (Kröger *et al.*, 2020). Individual differences between dogs in fermentation capacity of faecal microbiota were, however, found *in vitro* (Bosch *et al.*, 2016) suggesting that, depending on the intestinal microbiota present, undigested parts of insects might be fermented.

With this study we aimed to evaluate the effects of including BSFL meal in a dry extruded food on nutritional quality and some health aspects in healthy adult cats. Specifically, we measured food acceptance, apparent total tract digestibility, faecal consistency, fermentation products and microbiota, and serum biochemistry and haematology. It was hypothesised that feeding the BSFL meal-based dry extruded food to healthy adult cats for 4 weeks would result in shifts in faecal microbiota and similar values for the other read-out parameters as measured relative to feeding a poultry meal-based food.

2 Materials and methods

The present study was performed under a project license (project number 2017.W-0073, AVD number AVD1040020174324) issued by the competent national authorities (CCD, Central Committee for Animal Experiments). Approval to start was obtained after assessment of the detailed study protocol (experiment number 2017.W-0073.007) by the institutional animal welfare body (AWB) of Wageningen University (in Dutch: Instantie voor Dierenwelzijn, IvD) in accordance with European Union Directive 2010/63.

Experimental design

Two dry extruded foods were formulated to meet the FEDIAF (2022) nutrition guidelines for adult cats of which one was poultry meal-based (control) and the other BSFL meal-based. Foods were manufactured using processing in line with commercial pet foods assuring relevance of study outcomes for practice. Foods were fed to the two groups of four cats in a cross-over design with two 28-day periods and sample collection during the last 7 days of each period. Cats were assigned to one of these two groups based on sex and body weight. Biotechnicians were blinded to the foods offered. Read-out parameters included food acceptance, apparent total tract nutrient digestibility, faecal consistency, fermentation products and microbiota, and serum biochemistry and haematology.

Animals, housing and care

Adult purpose-bred European short-hair cats (four males, four females; 3.8-5.2 kg BW; 2.3-y.o.; castrated/sterilised) were assigned to one of two groups based on sex and BW with Group 1 and Group 2 having an average BW of 4.3 and 4.7 kg, respectively. All cats were weighed, scored for body condition on a 9-point scale (Laflamme, 1997) and assessed for various health parameters (i.e. muscle condition, eye and ear health, oral cavity health, coat condition, chest auscultation, locomotion) prior to the start of the study. Based on these scores, all cats were considered to be in good health. Cats were housed in groups of the same sex in free-living rooms during the first 21 days of each period. During the last 7 days of each period, cats were housed individually in metabolic cages ($0.80 \times 1.00 \times 0.75$ m) to allow adaptation and execution of individual faeces collection with removable litter boxes containing polyethylene grains (diameter 2 to 4 mm) (for details, see Van Rooijen *et al.*, 2016). During these days, cats were socialised with a familiar person for 30 min in the morning and 30 min in

the afternoon in a group under constant supervision to ensure identification of any faeces voided. To assure cats readily defecated in the litter boxes with polyethylene grains, cats were adapted to these boxes for 4 days prior the faeces collection period. General health (physical appearance, behaviour) of cats was checked daily by biotechnicians. Body weight, body condition and health scores were recorded every 2 weeks throughout the study.

Foods and feeding

Two dry extruded foods were formulated to meet the FEDIAF guidelines (2021) for adult cats. The control food consisted of poultry meal (34.3%), oat groats (20.0%), peas (14.0%), corn (10.0%), olive oil (6.2%), dried potato (5.0%), flaxseed (2.0%), digest (2.0%), premix (1.5%), lignocellulose (1.5%), stress mixture (1.0%), sodium chloride (0.5%), dried tomato (0.5%), potassium chloride (0.5%), herb, fruit and vegetable mix (0.25%), inulin (0.2), seaweed (0.2%), choline chloride (0.2%), cranberry (0.1%), and yucca (0.05%). The test food was formulated to be isoenergetic and was similar in composition, except for replacement of low-ash poultry meal (34.3%), olive oil (1.7%) and lignocellulose (1.5%) by 37.5% black soldier fly larvae meal (95.0% dry matter, 8.5% nitrogen, 14.3% crude fat, 6.0% ash; Protix, Dongen, The Netherlands). Foods were extruded by Jonker Petfood (United Petfood Group, Waalwijk, The Netherlands). Cats were fed individually in two equal meals around 7:30 and 15:30 for 30 min in their metabolic cage. Feeding levels were set at a level to maintain stable body weight based on historical feeding records and adjusted in cases required based on body weight measurements throughout the study. Food leftovers of the morning were weighed and added to the afternoon meal and total food intake was determined each day by weighing leftovers after the afternoon meal (acceptance). Water was made available *ad libitum* throughout the trial.

Faeces collection and analyses

To facilitate collection of freshly voided faeces, litter boxes were removed in the evening before collection. Clean boxes were placed the next morning before feeding after which these were monitored every hour. To allow the evaluation of faecal microbiota, grains in the litter box and tools for processing the faeces were sterilised with 70%-ethanol the day before and prior processing, respectively. In case of fresh faeces, consistency was scored on a scale from 1 to 5 by a biotechnician according to the Waltham system (Moxham, 2001) and polyethylene grains were removed. Faeces were then weighed, homogenised, subsampled for microbiota analyses and then stored at –20 °C pending further processing and analyses of dry matter (DM), short-chain fatty acids (SCFA), ammonia and biogenic amines. Each subsample for microbiota analyses was snap-frozen in liquid nitrogen and then stored in –80 °C pending further processing. Faeces processing and sampling was typically finished within 30 min after voiding. Once a sample of fresh faeces was collected for a cat, the removal of litter boxes and the hourly monitoring was not performed anymore for that cat but collection of faeces continued. All faeces produced were scored for consistency and cleared from polyethylene grains. Faeces were then weighed and stored at –20 °C pending further processing and analyses.

Faeces collected fresh and stored frozen were thawed, homogenised and subsampled for the respective analyses. For each cat and each period, a faecal sample (~1 g) was freeze-dried to a constant weight and DM content was based on moisture loss. Samples for SCFA (~1 g) were acidified with 2 ml phosphoric acid and isocaproic acid as internal standard, for ammonia (~1 g) with 2 ml trichloroacetic acid, and for biogenic amines (~1 g) with 2 ml 2% sulfosalicylic acid in 0.1 M hydrochloric acid. Analyses of SCFA and ammonia were performed as described in Eertink *et al.* (2020) and biogenic amines as in Saarinen (2002). The faecal DM content was used to calculate SCFA, ammonia and biogenic amine content in the original faeces.

For nutrient digestibility, faeces were pooled per cat (including leftover fresh faeces), oven-dried at 60 °C for 3 days and ground in a centrifugal mill to pass a 1-mm screen (ZM 200, Retsch GmbH, Haan, Germany). Foods and faeces were analysed for dry matter (ISO, 1999b), ash (ISO, 2002), nitrogen (NEN-EN-ISO, 2008), fat after acid hydrolysis (ISO, 1999a), and gross energy (ISO, 1998). Foods were also analysed for starch (ISO, 2004) and total dietary fibre (AOAC, 2005). Organic matter (OM) was calculated as DM – crude ash content and crude protein as nitrogen content ×6.25. Apparent total tract digestibility (%) values for nutrients and energy were calculated the equation: [(nutrient intake (g/d) – faecal nutrient output (g/d)) / nutrient intake (g/d)] × 100%, with faecal output being calculated as weight of pooled faeces + weight of all fresh faecal samples.

Details of procedures for bacterial and fungal quantification are described in the Supplementary material. The alpha diversity index was used to estimate the microbiome diversity within microbial communi-

ties, and this analysis included observed richness, Shannon's diversity index, and Pielou's evenness. For the beta diversity measure, principal coordinate analyses were carried out with the vegan package after transformation in a Bray-Curtis dissimilarity matrix. Composition of the community was generated at phylum, order, family and genus levels.

Blood collection and analyses

In the morning on the last day of the collection period (days 28 and 56), a blood sample was collected after the overnight fast. Cats were shaved using an electric clipper the day before to minimise handling time when sampling for blood. Blood withdrawal of the cats was performed in a separate and familiar room in the same building by an experienced veterinarian, a veterinary assistant to handle the cats and a biotechnician familiar to the cats to alleviate any potential stress. Furthermore, to reduce the amount of stress, the blood withdrawal was not attempted more than three times for each individual at each sampling time. Blood (~4.5-ml) was collected via jugular venipuncture. Each sample was immediately brought to a different room to keep the veterinary room as quiet and calm as possible. Blood was divided over vacutainer tubes (Vacuette, Greiner Bio-One, Alphen aan den Rijn, The Netherlands) with ~1 ml transferred into a 1-ml K3EDTA tube and stored at room temperature, ~2 ml in a 2-ml lithium heparin tube and stored in ice water, and 0.5 ml in a 4-ml serum tube and stored in ice water following instructions of the University Veterinary Diagnostic Laboratory of Utrecht University (Utrecht, The Netherlands). The remaining 1 ml of blood was used as spare volume. The heparin tubes were centrifuged within 60 min after collection at 2,000 ×*g* for 15 min at room temperature and 1 ml plasma was transferred in a vial. The serum tubes were centrifuged after 60 min of storage at 2,000 ×*g* for 15 min at room temperature and 0.25 ml serum was transferred in a vial. All samples were then transported to the University Veterinary Diagnostic Laboratory for serum chemistry and for haematology analyses.

Statistical analyses

All data were analysed using the Mixed Models procedure of SAS (version 9.4; SAS Institute, Cary, NC, USA) with Food, Period and Food × Period as a fixed effects and Cat as a random effect. All the significant threshold of *P*-values or adjusted *P*-values were set at <0.05. Least squares means for both dietary treatments are presented in tables.

TABLE 1 Analysed chemical composition (% of DM) of the poultry meal-based (PM; control) and black soldier fly larvae meal-based (BSFL) dry extruded cat foods

Parameter	Food	
	PM	BSFL
Dry matter, % as is	93.5	93.4
Organic matter	92.1	93.3
Crude protein	37.0	31.6
Acid-hydrolysed fat	14.6	14.6
Starch	33.2	32.9
Total dietary fibre	15.5	18.1
Gross energy, MJ/100 g DM	2.12	2.14

3 Results

Food composition

Foods contained similar amounts of organic matter, acid-hydrolysed fat, starch and gross energy (Table 1). The BSFL meal-based food had a lower crude protein content and higher organic matter and total dietary fibre contents than the control food. The sums of the constituents was above 100% for both foods, which relate to analytical error associated with each chemical analysis performed.

Food intake, faecal output, faecal characteristics and apparent total tract digestibility

One cat vomited at the last day of period 1 when fed the control food. No other health problems (including coat and skin condition) were noted throughout the study. Both foods were well-accepted by the cats with only some leftovers on days 1 and 2 of the study (control food: n = 1, 1st and 2nd day, 19%; test food: n = 3; 1st day, 5, 17, and 33%). Groups had similar BW at the start of the study ($P = 0.336$). Food type did not impact BW ($P = 0.850$), though BW was slightly higher in Period 2 (55 g; $P = 0.006$, data not shown). Body weight change tended to be slightly different between foods (+0.98% for control food versus +0.15% for test food; $P = 0.073$). In Period 2, the cats gained BW (+1.3%) whereas in Period 1 cats lost BW (–0.13%) ($P = 0.011$). Body condition score remained the same for all cats throughout the study (data not shown). Food intake was higher when cats were fed the BSFL-based food ($P < 0.001$) (Table 2). Moreover, food intake was higher in Period 1 (55.8 g/d) than in Period 2 (55.1 g/d) ($P = 0.006$). Faecal output was higher when cats were fed the food with BSFL meal compared to the control food ($P < 0.05$). Cats had a higher faecal output and lower digestibility values in in Period 2 than in Period 1. Faecal dry mat-

TABLE 2 Food intake (g/d), faecal output (g/d), faecal characteristics, and apparent total tract digestibility (%) of adult healthy cats fed poultry meal-based (PM; control) and black soldier fly larvae meal-based (BSFL) dry extruded foods

Parameter	Food		SEM	*P*-value		
	PM	BSFL		Food (F)	Period (P)	F × P
Food intake	54.2	56.7	2.0	<0.001	0.006	0.407
Faecal output	14.4	27.9	1.0	<0.001	0.003	0.186
Faecal DM, %	42.9	27.4	1.2	<0.001	0.407	0.551
Faecal score[1]	2.3	2.5	0.1	0.130	0.444	0.769
Digestibility						
Dry matter	87.8	85.5	0.6	0.031	0.014	0.414
Organic matter	91.0	87.1	0.5	0.001	0.012	0.237
Nitrogen	89.0	85.1	0.6	0.004	0.019	0.202
Acid-hydrolysed fat	95.0	94.6	0.5	0.405	0.039	0.134
Energy	90.8	87.3	0.5	0.002	0.012	0.111

SEM = pooled standard error of the mean.

[1]According to the Waltham system (Moxham, 2001) with score 1 = hard dry and crumbly; 2 = well formed, does not leave a mark when picked up; 3 = moist beginning to lose form, leaving a definite mark when picked up; 4 = majority, if not all, the form is lost; 5 = watery diarrhoea.

ter was lower when cats were fed the food with BSFL meal compared to the control food ($P < 0.001$). Consistency scores of fresh faeces, however, were optimal and did not differ between the foods. Compared to the control food, the food with BSFL meal had lower apparent faecal digestibility values for dry matter, organic matter, nitrogen, and gross energy ($P < 0.05$) and similar fat digestibility ($P = 0.628$).

Faecal fermentation products

Feeding cats the food with BSFL meal resulted in changes in fermentation product concentrations (Table 3). For example, faecal ammonia was lower when cats fed this food compared to the control food ($P < 0.001$) but the faecal concentrations of the short-chain fatty acids and biogenic amines were generally increased ($P < 0.05$).

Faecal microbiota

Relative to feeding cats the control food, feeding BSFL meal-based food reduced the number of faecal bacterial species (~600 vs ~350, $P < 0.001$) and reduced the alpha diversity measures Shannon's diversity index ($P < 0.001$) and Pielou's evenness ($P = 0.001$; Figure 1A) and the variability in faecal microbial community composition ($P = 0.001$; Figure 1B) with large shifts in relative abundance of the top 10 genera (Figure 1C). Faecal fungi were not affected by the dietary treatment ($P > 0.10$; see Supplementary Tables S2 and S3, Supplementary Figure S1). Relative to feeding cats the control food, feeding BSFL meal-based food increased the relative contribution of *Bifidobacterium* ($P < 0.001$), *Megasphaera* ($P = 0.003$), *Catenilbacterium* ($P = 0.009$) and *Megamonas* ($P = 0.010$) and decreased these values for *Prevotella*_9 and *Peptoclostridium* (Table 4). More bacteria were affected when feeding the BSFL meal-based food including reductions in the family Lachnospiraceae and genera *Lachnoclostridium* and *Negativibacillus* ($P < 0.01$) (Supplementary Table S1).

Blood biochemistry and haematology

In Period 1, there was one cat that showed three times resistance to blood withdrawal resulting in failure of the attempt. The cat was placed back in the cage and a missing sample was accepted. Insufficient blood was collected from one cat in Period 2 for analyses of serum proteins. Furthermore, two tubes clotted rendering the samples unfit for haematology profile analyses. Ranges in observed values of the parameter for both treatments are shown in Supplementary Tables S4 and S5. Multiple biochemistry parameters were affected by food fed to the cats ($P < 0.05$; Table 5), but least squares means remained within the reference intervals (R.I.; University Veterinary Diagnostic Laboratory) except for albumin. Albumin concentrations were higher than the R.I. for all except two observations (both cats fed the BSFL meal-based food) and cats fed the control food had higher values than when they were fed the BSFL meal-based food ($P = 0.010$). Chloride concentrations were higher than the R.I. for all cats in both periods. The concentrations of α2-globulins and β1-globulins were higher than the R.I. in Period 1 for three cats (two fed control food,

TABLE 3 Faecal fermentation products (μmol/g DM) of adult healthy cats fed poultry meal-based (PM; control) and black soldier fly larvae meal-based (BSFL) dry extruded foods

Parameter	Food		SEM	*P*-value		
	PM	BSFL		Food (F)	Period (P)	F × P
Ammonia	94.6	70.3	8.0	0.074	0.403	0.641
Short-chain fatty acids						
Acetate	132	295	17	<0.001	0.398	0.458
Propionate	66	119	10	0.003	0.430	0.758
Butyrate	34	80	7	0.003	0.070	0.349
Iso-butyrate	4.1	3.3	0.5	0.114	0.801	0.666
Valerate	14.6	30.8	4.0	0.006	0.298	0.690
Iso-valerate	6.3	4.5	0.7	0.045	0.730	0.560
Total SCFA	232	493	28	<0.001	0.295	0.590
Total BCFA	25.3	38.0	4.8	0.029	0.404	0.867
BCP, %	10.0	7.2	1.0	0.035	0.780	0.958
Biogenic amines						
Agmatine	1.7	2.4	0.2	0.086	0.706	0.652
Putrescine	1.0	5.9	0.8	0.002	0.584	0.546
Cadaverine	5.0	11.6	1.8	0.003	0.842	0.572
Histamine	0.2	1.4	0.3	0.006	0.936	0.879
Tyramine	0.3	0.3	0.1	0.764	0.167	0.167
Spermidine	0.8	0.7	0.1	0.395	0.727	1.000
Tryptamine	2.0	1.7	0.3	0.276	0.487	0.481
Pyrrolidine	0.3	0.1	0.0	<0.001	1.000	0.705

SEM = Pooled standard error of the mean; SCFA = straight-chain fatty acids; BCFA = branched-chain fatty acids; BCP = branched-chain proportion.

one fed BSFL meal-based food) and in Period 2 for three cats (three fed control food, two fed BSFL meal-based food). For other parameters, values for a specific cat were occasionally outside the range, but without a clear pattern relating to either dietary treatment or period.

Food fed to the cats impacted only mean corpuscular volume and neutrophil values ($P < 0.05$) but least squares means were for both dietary treatments within the R.I. (Table 6). The concentrations of erythrocytes and haemoglobin were higher than the R.I. in Period 1 for, respectively, three cats (one fed control food, two fed BSFL meal-based food) and four cats (one fed control food, three fed BSFL meal-based food) and in Period 2 for six cats (four fed control food, two fed BSFL meal-based food) and all cats. For other parameters, values for a specific cat were occasionally outside the range, but without a clear pattern relating to either dietary treatment or period.

4 Discussion

Previous work has shown that BSFL-based dry extruded foods can, depending on insect meal inclusion level, be palatable, have nutrient digestibility in line with conventional protein sources, and support good faecal consistency (reviewed by Bosch and Swanson, 2021). The apparent faecal nitrogen digestibility for the BSFL meal-based food in the present study (85.1%) was higher than that reported in other studies evaluating foods with BSFL ranging from 73.4 to 79.8% for dry extruded foods (Bosch and Swanson, 2021) and 82.3% for a wet retorted food (Do *et al.*, 2022). Such differences in digestibility values mainly relate to variation in insect meal quality, variable meal inclusion rates in the foods and the presence of other protein-containing ingredients that also contribute to the observed faecal digestibility values. The lower digestibility of the BSFL meal-based food in comparison to the poultry meal-based food was likely related to the presence of low degradable nitrogenous compounds like chitin. Overall digestibility values for the BSFL meal-based food were still high and

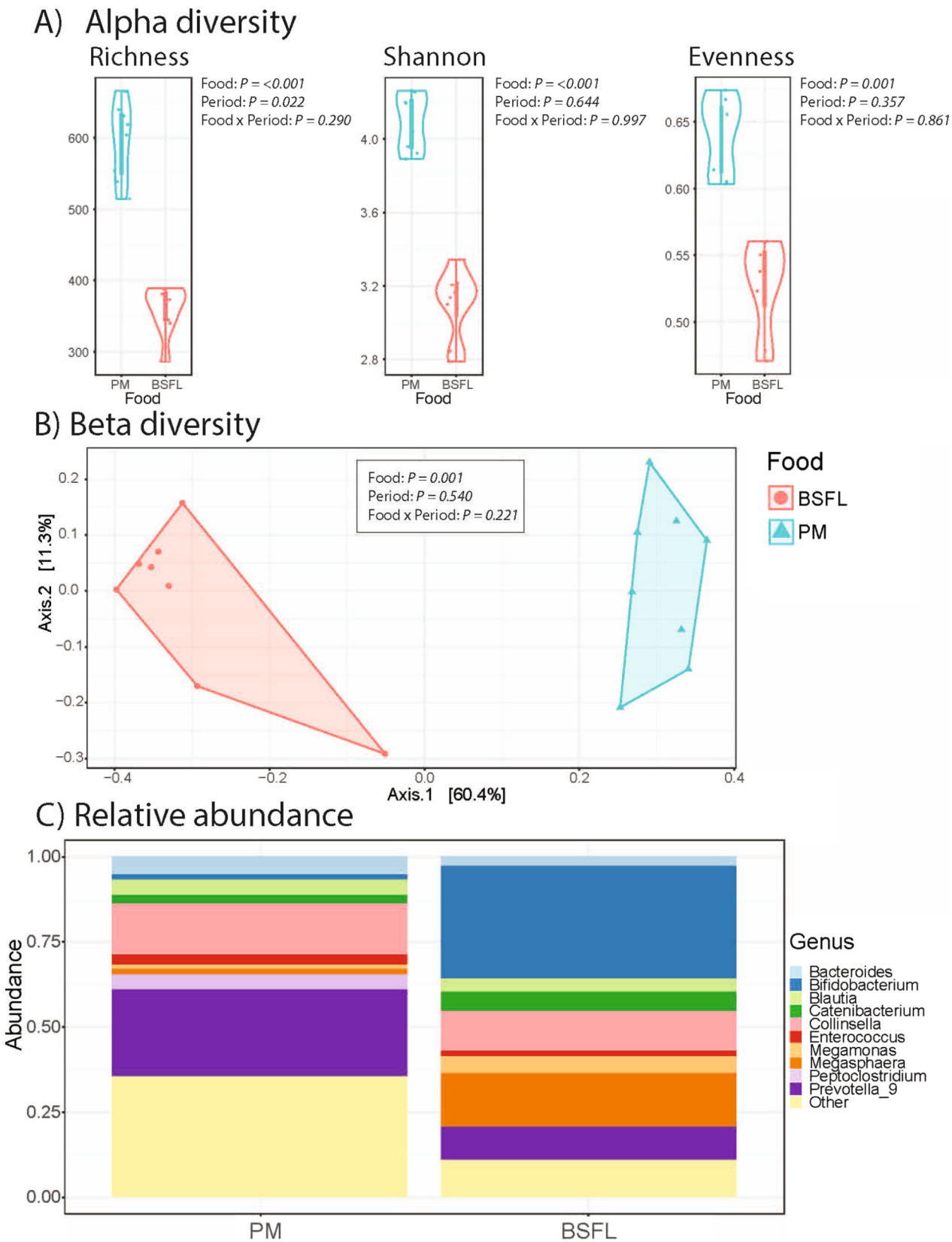

FIGURE 1 Faecal bacterial diversity and composition of adult healthy cats fed poultry meal-based (PM; control) and black soldier fly larvae meal-based (BSFL) dry extruded foods. Alpha diversity measures, observed richness, Shannon's diversity index, and Pielou's evenness, in BSFL (red) and PM (blue) (panel A). Beta diversity based on principal coordinate analysis using Bray-Curtis dissimilarity for BSFL (red) and PM (blue) (panel B). Relative abundance of the top 10 genera for BSFL (left) and PM (right), where each colour represents a different genus (panel C).

TABLE 4 Relative abundance of the top 10 bacterial genera in faeces of adult healthy cats when fed a poultry meal-based (PM; control) and a black soldier fly larvae meal-based (BSFL) dry extruded food

Genera	Food		SEM	*P*-value		
	PM	BSFL		Food (F)	Period (P)	F × P
Bacteroides	0.055	0.030	0.011	0.025	0.581	0.915
Bifidobacterium	0.016	0.329	0.023	<0.001	0.322	0.473
Blautia	0.046	0.039	0.009	0.542	0.230	0.293
Catenibacterium	0.024	0.058	0.009	0.015	0.634	0.372
Collinsella	0.150	0.118	0.017	0.236	0.687	0.549
Enterococcus	0.029	0.016	0.016	0.554	0.152	0.348
Megamonas	0.012	0.049	0.008	0.010	0.837	0.619
Megasphaera	0.016	0.157	0.231	0.003	0.510	0.608
Peptoclostridium	0.045	0.001	0.008	0.006	0.553	0.583
Prevotella_9	0.259	0.097	0.035	0.017	0.789	0.621

SEM = pooled standard error of mean.

TABLE 5 Serum chemistry profiles of adult healthy cats fed poultry meal-based (PM; control) and black soldier fly larvae meal-based (BSFL) dry extruded foods

Parameter	Food		SEM	*P*-value			R.I.
	PM	BSFL		Food	Period	F × P	
CREA, mmol/l	145	134	5	0.020	0.280	0.200	76-164
Urea, mmol/l	7.0	6.3	0.2	0.010	0.051	0.652	6.1-12.8
Glucose, mmol/l	4.9	5.0	0.3	0.261	0.084	0.944	3.4-5.7
TP, g/l	68	63	1	0.009	0.040	0.735	54-70
Albumin, g/l	43	37	1	0.006	0.089	0.938	25-34
α1-Globulin, g/l	2	2	0	–	–	–	2-5
α2-Globulin, g/l	4	4	0	0.219	0.664	0.737	4-7
β1-Globulin, g/l	7	7	0	0.667	0.667	0.733	4-7
β2-Globulin, g/l	5	6	0	0.056	0.018	0.944	4-9
γ-Globulin, g/l	8	7	1	0.346	0.346	0.775	4-8
Ca, mmol/l	2.60	2.49	0.03	0.007	0.006	0.437	2.36-2.86
P, mmol/l	1.47	1.45	0.06	0.831	0.696	0.933	0.89-2.05
Mg, mmol/l	0.89	0.89	0.02	0.843	0.048	0.767	N.A.
Na, mmol/l	154	153	0	0.070	0.011	0.041	146-158
K, mmol/l	3.8	4.2	0.1	0.005	0.546	0.218	3.4-5.2
Cl, mmol/l	119	120	0	0.318	0.687	0.450	105-112
ALP, U/l	31	32	2	0.397	0.020	0.862	15-63
ALAT, U/l	88	70	8	0.065	0.545	0.257	39-95
ASAT, U/l	27	23	2	0.076	0.028	0.686	N.A.
TBIL, μmol/l	2.6	2.2	0.1	0.004	0.023	0.182	0-3
CHOL, mmol/l	4.3	4.8	0.3	0.028	0.040	0.691	N.A.
TG, mmol/l	0.32	0.33	0.02	0.636	0.198	0.768	N.A.

SEM = pooled standard error of mean; R.I. = reference interval (UVDL, 2020); CREA = creatinine; TP = total protein; ALP = alkaline phosphatase; ALAT = alanine aminotransferase; ASAT = aspartate aminotransferase; GGT = gamma glutamyl transferase; TBIL = total bilirubin; CHOL = cholesterol; TG = triglycerides; N.A. = reference interval is not available.

Period 1, PM n = 3 and BSFL n = 4; Period 2, PM n = 4 and BSFL n = 3 for serum proteins and BSFL n = 4 for other parameters.

TABLE 6 Haematology profiles of adult healthy cats fed poultry meal-based (PM; control) and black soldier fly larvae meal-based (BSFL) dry extruded foods

Parameter	Food		SEM	*P*-value			R.I.
	PM	BSFL		Food (F)	Period (P)	F × P	
Erythrocytes, $\times 10^9$/l	10.3	10.1	0.3	0.454	0.604	0.392	6.0-10.0
Haemoglobin, mmol/l	8.9	9.0	0.2	0.706	0.114	0.536	5.0-8.0
Haematocrit, %	41	41	1	0.521	0.063	0.486	28-47
MCV, fl	40	41	1	0.048	0.019	0.413	37-55
MCH, fmol	0.88	0.89	0.02	0.288	0.079	0.378	0.71-1.07
MCHC, mmol/l	21.7	21.5	0.1	0.253	0.334	0.850	16.3-22.3
Thrombocytes, $\times 10^9$/l	155	204	45	0.421	0.935	0.568	156-626
Lymphocyte, $\times 10^9$/l	5.5	5.2	0.7	0.310	0.919	0.340	2.0-7.2
Monocyte, $\times 10^9$/l	0.3	0.3	0.0	0.949	0.055	0.533	0.0-1.0
Eosinophil, $\times 10^9$/l	0.9	0.9	0.1	0.980	0.642	0.800	0.3-1.7
Basophil, $\times 10^9$/l	0.0	0.0	0.0	0.508	0.508	0.482	0.0-0.1
Leucocytes, $\times 10^9$/l	12.0	13.2	1.0	0.205	0.284	0.740	6.3-19.6
Neutrophil, $\times 10^9$/l	5.3	6.9	0.5	0.044	0.097	0.264	3.0-13.4

SEM = pooled standard error of mean; R.I. = reference interval (UVDL, 2020); MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; WBC = white blood cell count.
Period 1, PM n = 2 and BSFL n = 3; Period 2, PM n = 4 and BSFL n = 3.

considered nutritionally suitable based on commonly accepted guidelines (FEDIAF, 2022).

The BSFL meal-based food contained, however, undigestible but still fermentable compounds with a profound impact on the intestinal microbial activity and composition as illustrated by the faecal profiles of fermentation products and microbial indicators of diversity and species. This is a novel insight and was unexpected based on low *in vitro* fermentability of undigested residues using feline faecal microbiota (Bosch and Post, 2019) and faecal fermentation products in dogs fed a BSFL meal-based extruded food for 35 days (Kröger *et al.*, 2020). Though studies may vary in faecal fermentation product concentrations, our results for increased faecal straight-chain fatty acids align with those in cats fed a dry extruded food with non-fermentable cellulose (270 μmol/g DM) and fermentable pectin (494 μmol/g DM) (Barry *et al.*, 2010). Faecal ammonia and total biogenic amines, which are products of amino acid fermentation (Macfarlane and Macfarlane, 2012) and were lower in concentrations than reported previously (Barry *et al.*, 2010), were, respectively, decreased and increased by including the BSFL meal, which suggests increased ammonia use for microbial protein synthesis but also some increased protein fermentation. The undigestible but still fermentable compounds seemed to steer the microbiota by acting as substrates for *Bifidobacterium*, *Megasphaera*, *Catenibacterium* and *Megamonas*. Increased relative abundance of these bacteria genus is associated to complex carbohydrates fermentation and production of straight-chain fatty acids, considered beneficial for host health (Alessandri *et al.*, 2020; Ganz *et al.*, 2022). *Catenibacterium* and *Megasphaera* are highly correlated with propionate production in the mammalian intestine, whereas *Megamonas* is associated to butyrate production (Butowski *et al.*, 2019). Interestingly, significant reduction in *Lachnoclostridium* and absence of *Negativibacillus* was observed when cats received BSFL meal-based diet, those genus were previously associated to digestive issues and negative for gut health (Jian *et al.*, 2022; Rojas *et al.*, 2023). However, a higher microbial diversity, generally considered to be favorable for health, was observed for the control treatment. The impact of these changes in microbial activity and composition when cats were fed the BSFL meal-based food for gut health warrant further study. Moreover, the higher hindgut fermentation activity in the cats fed BSFL meal-based food likely explains the associated (osmosis-induced) higher faecal moisture and daily faecal output. Faeces also remained to be well-formed with optimal consistency score, which suggests enhanced water binding capacity. Whether these effects are functional in cats with constipation also warrant further study.

Evaluation of serum biochemistry and haematology did not reveal clinically relevant dietary treat-

ment effects on cat health, which is in agreement with previous work (Pezzali and Shoveller, 2021; Do *et al.*, 2022). The least squares means for all serum biochemistry and haematology parameters measured were within the R.I. provided by the diagnostic laboratory, with the exception of albumin, chloride, erythrocytes and haemoglobin. The albumin, erythrocyte and haemoglobin concentrations measured above the R.I. were likely caused by relative increases in relation to plasma water due to mild haemoconcentration resulting from mild dehydration, not clinically significant at the levels reported (Cornell University College of Veterinary Medicine, 2023). Chloride measured mildly above the provided R.I. for all samples in the study period (118-120 mmol/l; R.I. 105-112 mmol/l) and no clinical cause for this was evident from the study; it should be noted that the provided R.I. from UVDL is lower and more narrow than other leading global veterinary diagnostic laboratories (111-124 mmol/l, Cornell University, 2023; 117-126 mmol/l, University of California Davis, 2023; 114-123 mmol/l, University of Guelph, 2023). Several parameters were outside the R.I. for individual cats also without clear clinical relevance. For example, glucose was slightly above the interval for one individual while on the test diet (6.4 vs 3.4-5.7 mmol/l), which was likely caused by the attempts to sample blood resulting in mild stress hyperglycaemia (Rand *et al.*, 2002). Thrombocytes measured below the R.I for five samples (automated count methodology, Laser-based, Siemens). A manual blood film count was not performed but could have given insight in thrombocyte aggregation as a cause of low thrombocyte levels. True feline thrombocytopenia is, however, very rare (Norman *et al* 2001) and there were no clinical signs of low thrombocyte levels (i.e. petechia, ecchymosis, haematuria, lethargy etc) reported in any of the study cohort individuals hence the below R.I. Thrombocyte levels are assumed to be artefactual.

Overall and based on the various parameters measured, this study showed that a BSFL meal-based extruded dry food is readily accepted by healthy adult cats, yields optimal faecal consistency and can support their health when fed for 28 days. Haematology and serum biochemistry parameters that were found to be different between the two tested foods and those of individual cats that were outside reference intervals were deemed not clinically relevant. Inclusion of BSFL meal increased faecal output and reduced apparent faecal digestibility values for dry matter, organic matter, nitrogen, and gross energy, likely due to low degradable compounds like chitin. Yet, both control and test foods presented high nutrient digestibility levels which are considered suitable based on commonly accepted nutritional guidelines. Based on faecal fermentation products, microbiota activity was enhanced when cats were fed the BSFL meal-based food, which was also confirmed by higher abundance of relevant genera of fermentative bacteria. Moreover, reduced faecal microbial diversity suggests that inclusion of BSFL meal steered the microbial composition in the distal gut of cats. This study therefore provides new leads for investigations into the impacts of BSFL dietary inclusion into feline gut health.

Supplementary material

Supplementary material is available online at:
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Conflict of interest

B.A.L. is employed by Protix, A.P. was employed by Protix and N.S. is employed by Petgood AB. The other authors have no conflict of interest.

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