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# COMPANION ANIMAL NUTRITION

# Short communication: the effects of a semi-synthetic diet with inclusion of black soldier fly larvae meal on health parameters of healthy adult cats

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# Abstract

In recent years, black soldier fly larvae meal (Hermetia illucens; BSFLM) has gained attention as a high value alternative protein source that is of great interest to the pet food industry. However, little is known regarding the effects of BSFLM on health parameters in adult cats. Thus, the objective of the current study was to determine the short-term effects of a semi-synthetic diet containing 4.6% inclusion of BSFLM on complete blood count (CBC) and serum biochemistry profile of healthy adult cats. Healthy adult male cats (n = 8; 1.4 yr) were fed the experimental diet for 21 d (experimental period) to maintain BW. Cats were washed in on a commercial diet and blood samples were collected before the start and at the end of the experimental period to measure gross health parameters. Results were analyzed as one-way ANOVA using the GLIMMIX procedure in SAS with cat as a random effect (SAS v. 9.4, The SAS Institute, Cary, NC). Cats lost an average of 5% of their BW (P = 0.0003) due to a concurrent decrease in food intake. A significant increase of alanine aminotransferase, chloride, potassium, sodium, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration was observed on day 21 vs. baseline (P < 0.05). In contrast, albumin, amylase, calcium, cholesterol, eosinophil, lymphocyte, monocyte, mean platelet volume, red blood cells, total protein, total solid proteins, and urea decreased over time (P < 0.05). However, all CBC and serum biochemistry parameters stayed within reference range for adult cats, with exception of glucose and mean corpuscular hemoglobin concentration that were above and below the reference range, respectively. Transient increases in glucose concentrations were likely due to sedation with dexmedetomidine prior to blood collection. The changes observed over time in the aforementioned parameters are likely due to changes in macronutrient composition of the diets offered prior to and during the experimental period (commercial diet vs. semi-synthetic diet, respectively) and cannot be attributed solely to a unique property of BSFLM. In conclusion, cats fed a semi-synthetic diet containing 4% BSFLM inclusion for 21 d remained healthy with no clinically relevant changes in CBC and serum biochemistry parameters. Further research should focus on longer term feeding studies and the ability of BSFLM to support the health and well-being of cats.

Key words: cat, insect meal, safety, semi-purified diet

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#### Abbreviations

ALT	alanine aminotransferase
BSF	black soldier fly
СНО	cholesterol
CBC	complete blood count
MCHC	mean corpuscular hemoglobin
	concentration
MCH	mean corpuscular hemoglobin
MCV	mean corpuscular volume
MPV	mean platelet volume
RBC	red blood cell
TP	total protein
TSP	total solid proteins

# Introduction

The growth of the pet food industry is driven by the use of novel ingredients and food forms, and the development of final food products that meet not only nutrient requirements, but also consumer demand. With the projected increase in human and pet populations, the use of more environmentally sustainable ingredients that do not directly compete with the human food sector will be essential to keep the pet food industry sustainable. Because of these factors, the use of insect-derived ingredients has gained attention (Bosch and Swanson, 2021). Generally, edible insects have a higher feed conversion rate and require less land to produce larger yields of protein compared to traditional protein sources (Oonincx et al., 2015), and have the capacity to grow on a number of different substrates, including food waste (Sánchez-Muros et al., 2014). Furthermore, insect production systems have a small ecological footprint and produce less ammonia and greenhouse gases (Oonincx et al., 2010), and are less water intensive compared to animal production systems (van Huis, et al., 2013). However, unlike in the human food industry, meat co-products are highly used in pet foods, which were estimated to have a lower environmental impact than BSFL (Koukouna and Broekema, 2017). Thus, claiming insects are a sustainable protein source will depend on the benchmark of comparison and how sustainability is being quantified (Bosch and Swanson, 2021).

Although it is evident that environmental sustainability is a pivotal aspect to consider for future petfood ingredient procurement, characterization of the nutritional quality and safety of insect-based ingredients to establish their use in foods for companion animals is needed. The nutrient composition of insect-based ingredients varies widely depending on the species, substrate, life stage, and rearing conditions (Finke and Oonincx, 2014). Generally, edible insects present with relatively high crude protein content and methionine and cysteine are generally the first-limiting amino acids (Bosch et al., 2014). Furthermore, the high protein quality of black soldier fly larvae (BSFL; Do et al., 2020), black soldier fly (Hermetia illucens, BSF), housefly (Musca domestica), and yellow mealworm (Tenebrio molitor) has been reported previously (Bosch et al., 2016). A recent in vivo study reported that the inclusion of BSFL meal (BSFLM) up to 20% did not impact acceptance, health parameters, and the apparent total tract digestibility of amino acids in adult dogs (Freel et al., 2021). Similarly, inclusion of cricket meal up to 24% in dog diets did not affect health parameters; however, it decreased the apparent total tract digestibility of all nutrients assessed (Kilburn et al., 2020).

Although efforts have been made to evaluate the use of insect-based ingredients in dog diets, there is a paucity

of information regarding their safety in cat foods. The inclusion of different insect meals in retorted (4% inclusion rate; Hu 2020) and extruded diets (15% inclusion rate; Lisenko et al., 2018) did not negatively impact acceptance and apparent total tract digestibility of macronutrients in adult cats. However, the effects of those insect-based diets on health parameters have not been investigated in the aforementioned studies. To the best of our knowledge, there are no published studies evaluating the safety of using insect meal in cat foods. Thus, the aim of our study was to investigate the effects of feeding a semi-synthetic diet containing 4% BSFLM on complete blood count (CBC) and serum biochemistry parameters of adult cats for 21 d. We hypothesized that the short-term feeding of a semisynthetic diet with inclusion of 4% BSFLM would not lead to changes in the measures of CBC and biochemistry.

### **Material and Methods**

The study was completed at the University of Guelph during the fall of 2020 and conducted according to the guidelines for animal care and use provided by the American Veterinary Medical Association, the Canadian Council on Animal Care, and the Canadian Veterinary Medical Association. All ethical and animal related aspects of the experiment were approved by the University of Guelph Animal Care Committee (AUP#4424).

## Animals and Housing

Healthy adult neutered cats (n = 8) of similar age (18 mo) were used in this study. Purpose bred cats (Marshall Biosciences, North Rose, NY, USA) were group housed in a free-living environment and were kept in individual crates only during feeding. The room was approved for cat inhabitation by the Chief Veterinary Inspector of the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) under the Animals for Research Act prior to the arrival of the cats. The light (12h light:12h cycle), temperature (20 °C), and humidity (40%-60%) were controlled and monitored daily. Environmental enrichment was provided in the form of toys, scratching posts, hide boxes, perches, beds, and climbing apparatuses. Litter boxes and exterior surfaces were cleaned once daily prior to the morning feeding. Cats were socialized with a familiar individual 5 d a week, for 2 h each day. Socialization included the following activities: general health assessment, brushing, petting, voluntary play, and housekeeping.

#### **Experimental diet**

Cats transitioned from a commercial diet [T22 Total Grain-Free, Nutram Pet Products, Elmira, ON; Metabolizable energy = 3, 835 kcal/kg; Moisture= 10%, Crude protein (CP), min= 36%; Crude fat (CFat), min = 19%; Crude fiber (Cfiber), max = 5.5%] to the experimental diet over a 6-d period. The cats were offered 75% of their daily energy requirement as kibble and 25% as experimental diet for 2 d, followed by 50% kibble and 50% experimental diet for 2 d, and finally 25% kibble and 75% experimental diet for the last 2 d. After the transition period, the cats were fed 100% of their daily energy requirement from the experimental diet for 21 d (experimental period). The experimental diet consisted of a semi-synthetic diet formulated with 4% inclusion of BSFLM (Moisture = 4.95%; CP = 49.3%; CFat = 14.4%; Cfiber = 6.4%; Ash = 8.3%; EnviroFlight, LLC., Maysville, KY, USA). The experimental diet was prepared fresh daily as follows. Dry ingredients were mixed on a Kitchen-Aid (KitchenAid, St Joseph, MI, USA) at speed 4 for 3 min. Water was added onto the dry ingredients and mixed for an additional 3 min. Finally, the poultry fat was heated up to 40 °C, added to the batter, and mixed again for 3 min. Additional water was added to each cat's daily portion of food to improve the texture. The ingredient and nutritional composition of BSFLM and the experimental diet are presented in Tables 1 and 2, respectively. Cats were fed twice a day (0800 and 1600 h) to maintain body weight based on historical feeding records. Food was available for 1 h during each feeding, and fresh water was provided ad libitum in the room in multiple waters providing both still and running water.

 Table 1. Ingredient and nutrient composition of the semi-synthetic diet formulated with black soldier fly larvae meal

Ingredient	%, on as fed-basis
Wheat starch—pregelatinized	39.04
Water	23.08
Amino acid Premix <sup>1</sup>	10.81
Poultry Fat	10.00
Poultry Meal	5.00
Black Soldier Fly Larvae Meal	4.62
Cellulose	2.31
Dicalcium Phosphate	1.54
Palatant <sup>2</sup>	1.15
Potassium Chloride	0.77
Brewers yeast	0.77
SaltNaCl	0.38
Choline	0.31
Mineral Premix <sup>3</sup>	0.12
Vitamin Premix <sup>4</sup>	0.12
Nutrient Content	Analyzed content
ME, kcal/kg (calculated) <sup>5</sup>	2,992
DM, %	73.45
CP, %	21.77
Acid-hydrolyzed fat, %	16.60
Ash, %	5.94
Crude Fiber	1.36
Ala, %	1.91
Arg, %	1.35
Asn, %	0.93
Cys, %	0.25
Glu, %	4.54
Gly, %	1.76
His, %	0.53
Ile, %	0.80
Leu, %	1.45
Lys, %	0.89
Met, %	0.14
Phe, %	0.66
Trp, %	0.39
Tyr, %	1.75
Val. %	0.17

<sup>1</sup>Provides per 100 g of final diet: 2.62 g of L-glutamic acid, 1 g of L-tyrosine, 0.77 of L-alanine and glycine each, 0.69 g of L-arginine and L-leucine each, 0.62 g of proline, 0.54 g of L-lysine, L-threonine, and L-serine each, 0.38 g of L-valine, L-isoleucine and L-phenylalanine and L-asparagine H<sub>2</sub>O each, 0.23 g of L-histidine HCL H<sub>2</sub>O, 0.15 g of tryptophan, and 0.12 g of taurine. <sup>2</sup>PALASURANCE C45-140 Dry, Kemin Nutrisurance, Inc., USA. <sup>3</sup>Trouw Dog & Cat TM PMX 2016, Trouw Nutrition, Northwich, UK. <sup>4</sup>Trouw Cat Vit no K NG 2016, Trouw Nutrition, Northwich, UK. <sup>5</sup>Calculated metabolizable energy based on modified Atwater values.

# Body weight assessment, blood collection, and analysis

Throughout the study, clinical and behavior observations were performed daily by a veterinarian. The day before the transition period (baseline) and at the end of the experimental period (day 21), fasted blood samples (16 h) were collected and body weight was measured prior to the morning feeding. To reduce stress, cats were sedated with an intramuscular (IM) injection of dexmedetomidine (Zoetis Canada Inc., Kirkland, QC, Canada) (0.01 mg/kg) prior to blood collection. Blood was collected (~3 mL per tube) through medial saphenous venipuncture and a sample (~1 mL) was immediately transferred into a EDTA tube for analyses. The remaining (~2 mL) was transferred to heparin vacutainer tubes that were chilled on ice until they were centrifuged at 2,000  $\times$  *q* for 15 min and plasma was separated. Whole blood and plasma samples were immediately submitted to AHL (Animal Health Laboratory, Guelph, ON, Canada) for serum biochemistry and CBC analysis. After blood collection, sedation was reversed with atipamezole (ANTISEDAN, Zoetis Canada Inc., Kirkland, QC, Canada) (0.01 mg/kg).

## Statistical analysis

Serum biochemistry, CBC, and body weight data were analyzed using a one-way ANOVA using the GLIMMIX procedure in SAS (SAS v. 9.4, The SAS Institute, Cary, NC). Cat was considered a random effect and day as a fixed effect. Means were separated using Fisher's LSD. Results were considered significant at P < 0.05.

# **Results and Discussion**

Although the effects of BSFLM (Freel et al., 2021) and cricket meal (G. sigillatus; Kilburn et al., 2020) on health parameters when included in a standard formula have been previously investigated in dogs, to the best of our knowledge, this is the first study to examine the safety of BSFLM in adult cats. No abnormal clinical signs and behaviors were observed, and all cats were deemed healthy throughout the course of the study. However, due to the low palatability of the semi-synthetic diet, which resulted in concurrent decrease in food intake, cats lost an average of 5% (1.4%-7.8%) of their BW (P = 0.0003) between days 1 and 21 (5.24 + 0.59 vs. 4.99 + 0.65 kg, respectively). Three cats lost more than 6% of BW over the course of the study. The decrease in BW was a result of the unpalatable nature of semi-synthetic diets due to high inclusion of starch and synthetic amino acids and limited inclusion of protein meals. Cats tend to have greater feed intake when provided high protein/low carbohydrate diets in contrast to low protein/high carbohydrate (Salaun et al., 2017). The semisynthetic diet used in this study was formulated to be limiting in sulfur amino acids in order to be used in a follow-up study to determine the minimum methionine requirements in cats. Due to this, we were unable to increase the inclusion of protein meals and/or palatants with high concentrations of methionine and cysteine and thus compromised the palatability of the diet. Data regarding the development of the semi-synthetic use in this study and its effect on acceptability and behaviour were collected (data not shown), but are beyond the scope of this short-term study and will be reported in a separate manuscript.

The medium of all serum biochemistry (Table 2) and CBC (Table 3) parameters stayed within reference range for adult cats, with exception of glucose and mean corpuscular hemoglobin concentration (MCHC). Concentrations of glucose

Analyte, units <sup>1</sup>	Baseline	Day 21	SEM	P-value	Reference interval <sup>2</sup>
ALT. U/L	45.13(33–77)	49.75 (41–70)	3.78	0.0319	31–105
Albumin, g/L	36.38(35-40)	32.25(23–35)	1.09	0.0195	30-44
ALP, U/L	21.88(12-41)	26.38(16–36)	2.84	0.2817	12–60
Amylase, U/L	1017.13 (785–1246)	898.38(618–1134)	61.92	0.0062	482-1145
CK, U/L	296.37(133–766)	296.25(119–525)	61.88	0.5513	94–449
Ca, mmol/L	2.50(2.40-2.64)	2.43(2.38–2.54)	0.02	0.0294	2.22-2.78
Cl, mmol/L	114.25(112–117)	117.13(115–120)	0.59	0.0002	114–123
CHOL, mmol/L	5.70 (4.55–7.34	3.23(2.40-4.13)	0.27	0.0002	2–12
CRE, mmol/L	114.26 (85–130)	120.62(81–147)	7.60	0.3371	50-190
Globulin, g/L	30.88(27–37)	30.25(25-40)	1.80	0.5208	23-42
GLU, mmol/L	9.43(7.50–12.60)	8.06(5.00-11.60)	0.74	0.2152	4.4-7.7
Mg, mmol/L	0.78(0.70-0.90)	0.81(0.80-0.90)	0.02	0.1703	0.8-1.1
P, mmol/L	1.55(1.40 -1.74)	1.41(1.26–1.71)	0.05	0.0479	0.8-2.29
K, mmol/L	4.18(3.90-4.60)	4.43(4.00-4.80)	0.09	0.0673	3.6-5.2
Na, mmol/L	150(148–152)	151.87(150–153)	0.47	< 0.0001	147–157
TBIL, mmol/L	0(0–0)	0.13(0-1)	0.09	0.3343	0–3
TP, g/L	67.25(62–72)	62.50(58–73)	1.55	0.0101	60–82
Urea, mmol/L	7.15(6.40–8.30)	6.15 (4.70–8.50)	0.37	0.024	6–12

Table 2. Serum biochemistry profile of cats prior to and 21 d after consumption of a semi-synthetic diet formulated with 4% inclusion of BSFL meal

<sup>1</sup>Data are expressed as LSmean and range (min–max); ALT, alanine aminotransferase; ALP, Alkaline Phosphatase; CHOL, cholesterol; GLU, glucose; TBIL,Total Bilirubin; TP,Total protein.

<sup>2</sup>Reference interval provided by the Animal Health Laboratory–University of Guelph (Guelph, ON) based on 40 clinically healthy, fasted, adult cats.

decreased from baseline compared to day 21 (P < 0.05; Table 2), but were above the upper limit at both timepoints. Specifically, four cats presented glucose levels above the reference range on day 21. This was likely due to the use of dexmedetomidine as sedative prior to blood collection. Dexmedetomidine can result in significant transient increases in glucose concentrations post-administration in cats (Bouillon et al., 2020). The MCHC were slightly below the lower reference range at baseline, but increased over time and stayed within the reference range on day 21 (P < 0.05); which agrees with the increase in mean corpuscular hemoglobin (MCH) and the decrease in red blood cells (RBC) and mean corpuscular volume (MCV) on day 21 compared to baseline (P < 0.05). In one cat, MCHC was slightly above the reference range (353 vs. 350 g/L) on day 21.

A significant increase of alanine aminotransferase (ALT), chloride (Cl), potassium (K), and sodium (Na) was observed over time (P < 0.05). In contrast, a significant decrease was observed for albumin, amylase, calcium (Ca), cholesterol (CHO), eosinophil, lymphocyte, mean platelet volume (MPV), monocyte, total protein (TP), total solid proteins (TSP), and urea (P < 0.05) on day 21 compared to baseline. In four cats, urea levels were below the reference range on day 21, whereas one cat presented lower levels of TP and another cat presented lower levels of albumin on the same period. Although in average the CK, basophil, and monocytes values stayed within the reference range in day 21, for each parameter, one cat was above the reference range. Interestingly, although in three cats seg. neutrophils were below the reference range in day 21, it was above the reference range for one cat in the same period.

Most of those differences were minimal, not clinically relevant, and are probably a result of changes in dietary macronutrient composition rather than an effect of a compound specific to BSFLM. However, it is noteworthy that this was a short-term evaluation, and that those differences may become more clinically relevant in a long-term study and is a limitation of the current study. It is also important to mention that the reference range used is this based on 40 clinically healthy, fasted, adult cats, which were eating commercial diets. Although these reference ranges are clinically practical, it may not represent the normal range for cats eating a semi-synthetic diet due to the drastic changes in macronutrient composition. Thus, having some parameters slightly outside the reference ranges used in this study does not necessarily indicate abnormality. In the current study, the cats transitioned from a high-protein commercial diet (CP: 40%, dry-matter basis) to a semi-synthetic diet (CP: 21.77%, dry-matter basis) that was formulated to meet the CP recommended allowance for adult cats established by the National Research Council (NRC, 2006). The decrease in albumin, TP, TPS, and urea is likely a result of a metabolic adaptation to a decrease in protein and amino acid intake. In order to maintain nitrogen balance and conserve protein (nitrogen) content, the rate of protein turnover decreases in response to lower dietary intakes of protein (Scrimshaw and Young, 1989), explaining the lower concentrations of the aforementioned parameters. As health status was not compromised and the blood parameters were still within the reference range, one can assume that the protein intake was meeting the cats' requirement.

Similarly, the lower crude fat content in the semi-synthetic diet compared to the commercial diet (16.6% vs. 21.1%, drymatter basis) may help explain the decreased in blood CHOL. Plasma CHOL content is influenced by dietary cholesterol and fat intake (Dobenecker et al., 1998). Due to the lower fat and CP content of the semi-synthetic compared to the commercial diet, the former had a greater nitrogen-free extract content which is largely due to carbohydrate content. Higher intakes of carbohydrate can increase the blood concentrations of ALT (Porikos and Van Itallie, 1983; Purkins et al., 2004), which is in agreement with the findings observed herein. In a recent thesis from the University of Illinois Urbana-Champaign (Hu, 2020), adult cats were fed complete and balanced wet foods containing 0% or 4% of either speckled cockroach, madagascar hissing cockroach or superworm (Hu, 2020). Although the CBC and serum biochemistry parameters were not fully presented, the authors stated that no differences

Analyte, units1	Baseline	Day 21	P-value	SEM	Reference interval <sup>2</sup>
Lymphocyte, ×10^9/L	4.36 (2.33–6.90)	3.27(1.74–5.44)	0.021	0.49	1.1-8.1
Basophil, ×10^9/L	0.05 (0-0.19)	0.05(0-0.39)	0.9163	0.04	0.0-0.26
Eosinophil, ×10^9/L	0.86(0.6-1.25)	0.35(0.11-0.78)	0.0002	0.09	0.0-1.6
Hb, g/L	119(100–130)	119.38 (108–130)	0.9266	3.11	93–153
MCH, pg	13.75(11–15)	14.75(13–16)	0.0331	0.39	13–17
MCHC, g/L	314.13(267-325)	335.13 (319–353)	0.0167	5.46	317-350
MCV, fL	44.50(41–48)	43.63 (40–46)	0.0209	0.70	39–52
MPV, fL	17.08(12.6-22)	13.45(10.2-16.8)	0.0012	0.95	8 –21
Monocyte, ×10^9/L	0.38 (0.11-0.50)	0.26(0.09-0.58)	0.0488	0.06	0-0.5
Platelets, ×10^9/L	234.63 (143–389)	329 (71–458)	0.0862	43.99	93-514
RBC, ×10^9/L	8.56(7.80-9.10)	8.18 (7.00-8.70)	0.0401	0.17	6.2-10.6
RDW, %	14.74 (13.4–15.5)	14.69 (14.3–15.1)	0.8351	0.20	14–17
Seg. Neutrophil, ×10^9/L	3.93(1.11-5.88)	4.81(1.67-8.93)	0.279	0.73	2.1-8.3
TSP, g/L	79.13(74–84)	68.13(62–75)	<0.0001	1.41	60–80
WBC, ×10^9/L	9.53(5.30-12.40)	8.70 (4.5–11.6)	0.3914	0.88	4.2-13

Table 3. Hematology profile of cats prior to and 21 d after consumption of a semi-synthetic diet formulated with BSFL meal

<sup>1</sup>Data are expressed as LSmean and range (min–max); Hb, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cell count; MCV, mean corpuscular volume; MPV, mean platelet volume; RBC, red blood cell count; RDW=red cell distribution width; TSP, Total solids protein.

<sup>2</sup>Reference interval provided by the Animal Health Laboratory—University of Guelph (Guelph, ON) based on 40 clinically healthy, fasted, adult cats.

were observed for the serum biochemistry parameters among dietary treatments, with exception of creatinine and glucose. This lack of difference for the health parameters among dietary treatments is probably due to similar macronutrient composition between diets and no detrimental effect of the inclusion of insects in the dietary fed to cats. Freel et al (2021) and Kilburn et al (2020) also did not observe major changes in CBC and serum biochemistry variables of dogs fed diets containing BSFLM and cricket meal, respectively. These results in the dog highlight our hypothesis that the changes observed in the CBC and serum biochemistry in this study are a response to changes in macronutrient composition of the diets and not due to the inclusion of BSFL. Although the effects of macronutrient composition and BSFLM are confounded and cannot be individually interpreted, the results still indicate that even in a semi-synthetic diet application, the inclusion of BSFLM did not result in abnormal clinical signs, behavior changes, and concentration of whole blood and serum biomarkers. We included BFSLM at 4.6% which is similar to the inclusion of speckled cockroach (4%), madagascar hissing cockroach (4%), or superworm (4%) in retorted cat diets by Hu (2020). Thus, BFSML has potential to be included in retorted diets for cats in the same inclusion level used in our study with no negative impact on health status. The changes observed herein may also be a result of differences in the ingredient and micronutrient composition of a commercial diet vs. a semi-synthetic diet. We unfortunately did not analyze the micronutrient composition of the diets and it was impractical to evaluate the effects of every ingredient on health status as the diets have different base formulas; thus, we are unable to draw conclusions regarding these aspects.

Beyond the potential safety evaluated herein, BSFLM may have potential nutraceutical properties that should be further investigated in the domestic cat. Chitin, a polysaccharide present in the exoskeleton of insects, is indigestible by some mammals, and thus, may display immunomodulatory properties (Henry et al., 2018) and promote gut health through fermentation in the hind gut (Hu, 2020). Furthermore, BSFLM is high in lauric acid (12:0), a medium-chain fatty acid that has antimicrobial activity against gram-positive bacteria (Spranghers et al., 2018). Taken together, more research investigating the long-term effects of insect-based ingredients on cat health is warranted to evaluate their safety and potential beneficial properties and consequently promote their use in commercial cat foods.

#### Conclusion

Cats fed a semi-synthetic diet with 4% inclusion of BSFLM for 21 d displayed CBC and serum biochemistry parameters within the reference range for adult cats, with exception of glucose on both time points, which was an artifact of the sedation prior to blood collection. A significant increase of ALT, Cl, K, Na, MHC, MCHC was observed over time (P < 0.05). On the other hand, albumin, amylase, Ca, CHO, eosinophil, lymphocyte, monocyte, MPV, RBC, TP, TSP and urea decreased (P < 0.05) on day 21 compared to baseline. Those changes were not clinically relevant and are probably a result of changes in macronutrient composition of a commercial vs. a semi-synthetic diet rather than an effect of BSFLM. Long-term studies investigating the inclusion of insect-based ingredients in commercial cat foods are necessary to validate their safety in cat foods.

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## **Conflicts of Interest**

The authors declare that there are no conflict interest regarding the publication. However, it is noteworthy that A.K.S. was previously employed by P&G and Mars Pet Care, serves on the Scientific Advisory Board for Trouw Nutrition, and has received honoraria and research funding from various commodity groups, pet food manufacturers, and ingredient suppliers.

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