



RESEARCH ARTICLE

# Black soldier fly larvae meal-based dog food: does the inclusion level impact kibble characteristics and *in vitro* digestibility?

E.L. Santos Neto<sup>1\*</sup> , C. Baptista da Silva<sup>2</sup> , F. Van Bockstaele<sup>3</sup> , M. Hesta<sup>2</sup> , M. Eeckhout<sup>1</sup>  and G. Rolandelli<sup>1,3</sup> 

<sup>1</sup>Cereal, Bakery and Feed Technology Research Group, Department of Food Technology, Safety and Health, Faculty of Bioscience Engineering, Ghent University, Valentin Vaerwyckweg 1, 9000 Ghent, Belgium; <sup>2</sup>Department of Morphology, Imaging, Orthopaedics, Rehabilitation and Nutrition, Ghent University, Salisburylaan 133, 9860 Merelbeke, Belgium; <sup>3</sup>Food Structure and Function Research Group, Department of Food Technology, Safety and Health, Faculty of Bioscience Engineering, Ghent University, Valentin Vaerwyckweg 1, 9000 Ghent, Belgium; \*elias.leocadiodossantosneto@ugent.be

Received 22 December 2025 | Accepted 9 March 2026 | Published online 24 March 2026

## Abstract

Insects in pet food are already a reality, and insightful information is available on nutrition and health. However, studies on insect-based kibble quality remain scarce. Thus, the aim of this study was to investigate the effects of different black soldier fly larvae (BSFL) levels on kibble characteristics and *in vitro* digestibility. Six isonitrogenous dog foods were formulated following the European Pet Food Industry Federation (FEDIAF) guidelines: one control (0% BSFL) and five with increasing BSFL levels (20, 40, 60, 80 and 100%). All diets were extruded at the same conditions, and per diet, three samples were collected in a five-minute interval during extrusion. Data were tested for normality (Shapiro-wilk). Parametric data were analysed by ANOVA followed by Tukey's test, while non-parametric by Kruskal-Wallis, at a 95% probability. Increasing levels of BSFL affected pasting properties (PT) ( $P < 0.05$ ) of the raw mixtures, suggesting demised starch gelatinization. Also, higher water absorption index was observed ( $P < 0.001$ ) at higher levels of BSFL inclusion. Increasing BSFL lowered the expansion index, increased hardness ( $P < 0.001$ ), and changed in microstructure and darker kibbles ( $P < 0.001$ ). The inclusion of 100% BSFL lowered dry matter (DM) *in vitro* digestibility ( $P < 0.001$ ), compared to the other diets. Digestibility of crude protein (CP), however, was lower than control from 40% BSFL inclusion ( $P < 0.001$ ) onwards. Nonetheless, both DM and CP digestibility of all diets are within the advised minimum limits of FEDIAF. Lastly, multiple bands at different wavenumbers could be distinguished on the Fourier-transformed infrared spectrometer spectra (FT-IR) among diets. Regardless unfavourable effects of increasing BSFL inclusion levels on PT and kibble characteristics, all diets were successfully extruded. These effects did not compromise the kibble quality and *in vitro* digestibility. Thus, BSFL meal inclusion can be used in dog food at any inclusion level.

## Keywords

*Hermetia illucens* – insect-based pet food – insect protein – pet food processing

## 1 Introduction

In Europe, 139 million households have pets, totalling a 299 million animals' population in the EU, resulting in a pet food production of 9.1 million tonnes on 2024 (FEDIAF, 2025), which requires a massive consumption of raw materials. In this regard, the environmental concern about the production of conventional protein sources is pushing the pet food industry into alternatives to poultry, beef cattle and swine by-products (Kępińska-Pacelik and Biel, 2022), as the livestock sector is largely associated with environmental damage (Wanapat *et al.*, 2015) and climate change.

Insects have been suggested as promising ingredients for pet food. Their crude protein levels can be higher than soybean meal and close to that for other ingredients such as fish and poultry (Bosch *et al.*, 2014). Particularly, black soldier fly larvae (BSFL) (*Hermetia illucens*) has been reported as potential protein source (Sogari *et al.*, 2019) for dogs and cats. Overall, BSFL composition can vary in a range of 41 to 43% of crude protein, 17 to 34% of fat, 4 to 10% of crude fibre and 15 to 27% of ash (Makkar *et al.*, 2014). Moreover, its inclusion in pet food can go further, with potential beneficial effects for the pet's health (Valdés *et al.*, 2022) due to the presence of specific saturated fatty acids like the lauric acid (Makkar *et al.*, 2014), and a natural antioxidant activity (Bolat *et al.*, 2021).

Although the variation in the composition, insect-based diets have been considered high-quality and efficient. It has been shown that the crude protein digestibility of BSFL in dogs can range between 72.7 and 89.7% (Abd El-Wahab *et al.*, 2021; Bosch *et al.*, 2014; Jian *et al.*, 2022; Kröger *et al.*, 2020; Lei *et al.*, 2019), depending on the level of inclusion. It is important to notice that the insect exoskeleton consists of a large amount of chitin, which is a linear polymer of  $\beta$ -(1-4)N-acetyl-D-glucosamine units, structurally similar to cellulose (Finke, 2007) that can increase crude fibre levels, and potentially, interfering in the protein utilization (Longvah *et al.*, 2011).

When it comes to traditional pet food, extrusion cooking is one of the most applied processes worldwide for producing pet food. It provides sanitization, adequate gelatinization of starch, shape and texture of kibbles (Baller *et al.*, 2018). During the extrusion process, the mixed ingredients are exposed to thermal and mechanical energy and moisture, that generates shear, high temperature and pressure (Baller *et al.*, 2021; Loureiro *et al.*, 2024). These factors, when sufficient, lead to a loss of starch crystallinity, swelling and dis-

ruption, turning the mixture into a viscous melt that expands as it exits the die, defining the final texture of the product (Bill Kaelle *et al.*, 2024). Besides, extrusion can improve digestibility and utilization of the nutrients of the diet (Tran *et al.*, 2008). However, fibrous ingredients can have a negative impact on extrudates, increasing the density and decreasing the expansion, and this can also affect diet palatability (Donadelli *et al.*, 2021). This happens due to the highly polymerized structures of fibre (Souza *et al.*, 2023). As chitin has a very similar structure as cellulose, it is important to know whether it can have a similar impact on the extrusion process and the properties of the extrudates properties.

To the best of our knowledge, only one study was found providing insights on the effects of insects in pet food (Chen *et al.*, 2025a). The authors found that including insects such as BSFL meal or crickets in dog food may lead to undesirable kibble quality and extrusion impairment. However, these potential effects can be attributed to many factors: insect meal manufacture; other constituents of a formula; level of insect inclusion, processing conditions and insect species. Therefore, more research is needed. Thus, the aim of this study was to evaluate the effects of replacing poultry meal protein source by different BSFL levels on kibble mechanical and structural characteristics, and *in vitro* digestibility.

## 2 Materials and methods

### *Raw materials, experimental diets and their pasting properties*

Six isonitrogenous experimental diets were formulated to meet dog's nutritional requirements according to the European Pet Food Industry Federation (FEDIAF, 2024). The conventional and minor ingredients (maize flour, poultry meal and potato starch, beet pulp, minerals, mineral and vitamin premix, DL-methionine, and antioxidant – United Petfood Group, Ghent, Belgium) were used to balance the experimental diets replacing poultry meal by partially defatted BSFL meal (ProteinX – Protix, Dongen, The Netherlands) in 0% (control), 20%, 40%, 60%, 80%, 100%. As the BSFL meal (54% of crude protein (CP) and 18% of fat on dry matter (DM) basis) has less crude protein than poultry meal (68.5% of CP and 18% of fat on DM basis) and takes more space in the formula, formulation adjustments were made on the maize flour inclusion to prevent major crude protein discrepancies. The dry ingredients were ground in a cross-beater mill (Retsch SK1) using a

TABLE 1 Ingredients (%) and analysed chemical composition (% of dry matter) of control and BSFL diets

	Control	Inclusion of BSFL meal				
		20%	40%	60%	80%	100%
<b>Ingredients (%)</b>						
Maize flour	45.70	44.20	42.70	41.20	39.60	37.00
BSFL meal	–	6.60	13.30	20.00	26.60	33.40
Poultry meal	25.80	20.70	15.50	10.30	5.30	–
Native potato starch	11.20	11.20	11.20	11.20	11.20	11.20
Beet pulp	3.00	3.00	3.00	3.00	3.00	3.00
Mineral and vitamin premix	0.90	0.90	0.90	0.90	0.90	0.90
Potassium chloride	0.70	0.70	0.70	0.70	0.70	0.70
Monocalcium phosphate	0.70	0.70	0.70	0.70	0.70	1.80
Natural antioxidant	0.45	0.45	0.45	0.45	0.45	0.45
DL-methionine	0.45	0.45	0.45	0.45	0.45	0.45
Choline chloride	0.30	0.30	0.30	0.30	0.30	0.30
Common salt	0.30	0.30	0.30	0.30	0.30	0.30
Others	10.50	10.50	10.50	10.50	10.50	10.50
<b>Chemical composition (% of dry matter)</b>						
Moisture	8.52	8.71	9.37	10.04	11.26	11.06
Crude protein	26.96	26.62	26.48	26.27	26.20	25.74
Acid-hydrolysis ether extract	5.50	5.90	6.10	5.00	6.53	6.75
Ash	6.60	6.30	5.90	5.50	5.10	4.80
Crude fibre	1.09	1.86	2.20	2.66	3.60	4.61
Nitrogen-free extract (NFE) (%)	58.32	57.57	57.80	58.82	57.00	55.60
Chitin/chitosan (%)	–	0.43	0.67	1.29	1.37	2.11
Metabolizable energy (kcal/kg)	3820	3850	3880	3920	3940	3935

Premix: AMMIN 097 (MIAVIT), amount per kg of ingredient: crude protein: 21.2264%; crude ash, 52.1095%; calcium, 0.1155%; sodium, 0.0000%; magnesium, 5.3000%; chloride, 0.0722%; sulphur, 8.5239%; taurine, 300 000.0000 mg; citric acid, 1250.0000 mg; vitamin A, 4 000.000.0000 IU; vitamin D3, 350 000 0000 IU; vitamin E, 40 000.0000 mg; vitamin K3, 800.0000 mg; vitamin H (biotin), 40 000.0000 mcg; folic acid, 1000.0000 mg; vitamin B1 (thiamine mononitrate), 2000.0000 mg; vitamin B2, 1600.0000 mg; vitamin B6, 1600.0000 mg; vitamin B12 (HPLC)(cobalamin), 10 000.0000 mcg; niacinamide, 16 000.0000 mg; calcium-D-pantothenate, 3261.0000 mg; copper (sulphate), 1000.0000 mg; manganese (sulphate), 3000.0000 mg; zinc (sulphate), 20 000.0000 mg; iodine (calcium iodate), 300.0000 mg; selenium (coated Naselenite), 30.0000 mg; BHT-Antioxidant, 8275.0000 mg; propyl gallate, 700.0000 mg; sepiolite, 384 280 5000 mg. Natural oxidant: Loxidan (Kaelster Nutrition, Cuxhaven, Germany). Others: Poultry fat and palatant. NFE and metabolizable energy are estimates.

1 mm sieve and blended according to the respective formula (Table 1).

The different diets were normalized to a moisture content of 10% prior to analysis. A rheometer (Modular Compact Rheometer MCR 102, Anton Paar) equipped with a ST24-2D/2V/2 probe and a starch pasting cell was used for the evaluation of the pasting properties of the raw blends following the method reported by Wu *et al.* (2022), with modifications. Briefly, 3.2 g of sample was added with 10 ml distilled water and stirred at 960 rpm for 1 min. Then, the heating-cooling program included the following stages during which the rotation speed was fixed at 160 rpm. The suspension was first held at 50 °C for 1 min and then heated to 95 °C at a constant

rate of 5 °C/min. After being held at 95 °C for 5 min, the samples were cooled down to 50 °C, at 5 °C/min and finally kept at 50 °C for 2 min. Each formula was evaluated in triplicates, and the resulting curves were analysed using the RheoCompass® software (RheoCompass software, Anton Paar) to determine the pasting temperature (PT), peak viscosity (PV), holding strength viscosity (HSV), final viscosity (FV), breakdown viscosity (BV), and setback viscosity (SB).

#### **Extrusion process and chemical composition**

The mixtures were fed into the extruder by an automated gravimetric feeder (DDSR20 2.0, Kubota Brabender Technologie) and extruded using a co-rotating twin-

screw extruder (TwinLab-F 20/40D Brabender<sup>®</sup>) with a high shear screw configuration and a 3 mm die at the end. All mixtures were extruded under the same conditions (24% target moisture content; 700 rpm screw speed, 5 kg/h dry material feeding rate, 0.977 kg/h water rate, heating zone 1: 30 °C; heating zone 2: 50 °C; heating zone 3: 70 °C; heating zone 4: 90 °C; heating zone 5: 110 °C; zone 6: 130 °C). After extrusion, the kibbles were left to cool down and dry for 30 min and were then stored in plastic containers at room temperature until further analysis. No coating was performed.

Prior to further analysis, all products were milled at 1 mm using a knife mill (GRINDOMIX GM 200, Retsch). Proximate analyses for dry matter (DM) was done following ISO 6496 guidelines, ash ISO 5984, ether extract (EE) ISO 6492 (with pre-hydrolysis), and crude protein (CP; nitrogen  $\times$  6.25) content was determined following the ISO 16634-1. Chitin assessment was performed according to D'Hondt *et al.* (2020). Ingredients (%) and analysed chemical composition of control and BSFL diets are shown in Table 1.

### Characteristics of kibbles

Water activity, water solubility index, water absorption index and oil absorption index: Water activity ( $a_w$ ) was determined in milled samples using a dew-point water activity meter (LabMaster.aw, Novasina). All measurements were performed in five replicates. Water solubility index (WSI) and water absorption index (WAI) were determined according to Rolandelli *et al.* (2020). All grinded samples were weighed ( $1.25 \pm 0.004$  g) into 15 ml tubes and filled with distilled water up to 15 ml. The tubes were vortexed for 5 min and immediately centrifugated at  $3000 \times g$  for 10 min. Afterward, the supernatant was placed into aluminium cans and allowed to dry overnight at 105 °C. All measurements were done in five replicates and WSI (%) and WAI (g/g) were calculated as follows:

$$\text{WSI (\%)} = (\text{weight of dried supernatant} / \text{weight of dried sample}) \times 100 \quad (1)$$

$$\text{WAI (\%)} = (\text{weight of wet sediment} / \text{weight of dried sample}) \times 100 \quad (2)$$

Oil absorption index (OAI) was determined according to Liadakis *et al.* (1993). Ground samples were weighed ( $1 \pm 0.003$  g) in six replicates into graduated 15 ml tubes. Thereafter, 6 ml of corn oil was added to each sample. All tubes were vortexed for 1 min and left

to rest for 30 min, followed by centrifugation for 20 min at  $700 \times g$ . After centrifugation, the free oil volume was read and OAI (ml/g) was calculated as follows:

$$\text{OAI (ml/g)} = (V_{\text{oil}}/m_s) \quad (3)$$

Where  $V_{\text{oil}}$  is the volume of absorbed oil (ml) and  $m_s$  is the sample mass (g).

Colour and total colour change: Colour evaluation was performed in ground samples by measuring the CIELAB space parameters:  $L^*$  (whiteness/darkness),  $a^*$  (redness/greenness),  $b^*$  (yellowness/blueness) using a colorimeter (CR-400, Konica Minolta). The total colour change ( $\Delta E$ ) was calculated according to Jafari *et al.* (2017) as follows:

$$\Delta E = \sqrt{(L - L_0)^2 + (b - b_0)^2 + (a - a_0)^2} \quad (4)$$

where  $L_0$ ,  $b_0$  and  $a_0$  indicate the respective colour values of the control diet (0% BSFL).

Expansion index and hardness: For the expansion index (EI), the width of 60 kibbles was measured with a digital calliper, and the expansion index was calculated as the ratio between the kibble width and the die diameter (3 mm). Hardness determination was performed according to Manbeck *et al.* (2017), by using a texture analyser (TA.XTplus, Stable Micro Systems) with an SMS P/0.5R probe. A compression test was run with a pre-test and test speed of 1.0 mm/s, post-test speed of 10 mm/s, and 50% strain. Fifteen pieces of sample of each formulation were used for the measurement.

*In vitro* digestibility protocol: *In vitro* digestibility was assessed according to the method described by Biagi *et al.* (2016) with small adjustments. Samples were ground through 1 mm sieve and  $2.00 \pm 0.002$  g of each were weighed into 250 ml Erlenmeyer flasks in duplicate. For the gastric phase, 80 ml of pepsin solution (2 g/l in 0.075 M HCl; Pepsin: P7000, Sigma-Aldrich) containing gastric lipase (1 g/l; lipase from *Rhizopus*: 80612, Sigma-Aldrich) and two drops of chloramphenicol solution (250 mg/50 ml in 96% ethanol; Chloramphenicol: C0378, Sigma-Aldrich) were added to each flask. Flasks were incubated at 39 °C for 2 h in a shaking incubator (New Brunswick Innova<sup>®</sup> 44, Eppendorf) at 90 rpm. Enzymatic activity was then halted by placing the flasks in a -20 °C freezer for 5 min. For the intestinal phase, 80 ml of pancreatin solution (10 g/l; Pancreatin: P1750, Sigma-Aldrich; in phosphate buffer: Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O 35.61 g/l (71662; Sigma-Aldrich) and

$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  6.242 g/l (71500: Sigma-Aldrich), mixed at 84:16) was added to the cooled flasks together with bile salts at a final concentration of 25 g/l (bile salts: 48305, Sigma-Aldrich) and incubation continued under the same conditions for 4 h. Afterwards, enzyme activity was stopped, and the contents were filtered through a nylon gauze (37  $\mu\text{m}$  pore size, 24% permeability; Kabel Zaandam) using a Büchner apparatus connected to an electric vacuum pump according to Bosch *et al.* (2016). The residue was rinsed twice with 50 ml of 96% ethanol and 99.5% acetone to remove free sugars and fat. Samples were then dried overnight at 70 °C until constant weight, cooled in a desiccator, and weighed. DM and CP were determined according to the methods previously mentioned. The coefficient of *in vitro* digestibility (CIVdig (%)) was calculated as follows:

$$\text{CIVdig (\%)} = \frac{(\text{g nutrient before digestion} - \text{g nutrient after digestion})}{(\text{g nutrient before digestion})} \times 100 \quad (5)$$

#### **Cryo-scanning electron microscopy (Cryo-SEM)**

For microstructure visualization, kibbles were glued on a pre-tempered aluminium cryo-SEM stub and cut in half using a sharp knife. Further, samples were vitrified using a nitrogen slush and then transferred into the cryo-preparation chamber at -140 °C and under vacuum (PP3010T Cryo-SEM Preparation System, Quorum Technologies, Lewes, UK). Kibbles were sublimated at -90 °C for 30 min, coated with platinum for 90 s and further visualized using the JEOL JSM 7100F cryo-SEM at -140 °C and using a voltage of 3 keV. All micrographs were taken at a magnification of 100 $\times$  to allow direct comparisons.

#### **Fourier-transformed infrared spectroscopy (FTIR)**

Molecular associations and components interactions in grinded kibbles were analysed using a Fourier-transformed infrared spectrometer (VERTEX 70, Bruker Optics) equipped with an attenuated total reflectance (ATR) diamond cell. Spectra were collected after performing 20 scans in the 4000-400  $\text{cm}^{-1}$  range with a 2  $\text{cm}^{-1}$  resolution. The range between 1800 and 2500  $\text{cm}^{-1}$  was not considered for comparative analysis. Each formulation was analysed five times and results were obtained using OPUS 7.5 software from Bruker. Spectra were normalized and baseline corrected to allow direct comparisons among experimental diets.

#### **Experimental design and statistical analysis**

This study was conducted in a completely randomized design and data normality was checked using the Shapiro–Wilk test at 95% probability. Data with normal distribution were analysed through analysis of variance (ANOVA) with means compared by Tukey test at 95% probability. For non-normal data, Kruskal–Wallis was performed, also at 95% probability. All the statistical analyses were performed in RStudio (R version 4.3.1 from 2023) using the easyanova package for means comparison.

### **3 Results**

#### **Pasting properties of raw formulations**

The pasting curves of the different raw experimental diets with increasing levels of BSFL meal are shown in Figure 1. From these curves the pasting parameters were calculated, and results can be seen in Table 2. There were no significant differences among diets for PT ( $P = 0.909$ ). On the contrary, PV progressively diminished with increasing levels of BSFL, although they were only significantly different from the control ( $P < 0.05$ ) at 80% and 100% BSFL meal inclusion. HSV was similar between the control and 20% BSFL inclusion. However, it was significantly reduced from 40% onward, with no significant differences among 40% to 100%, indicating lower starch integrity at higher BSFL meal levels. BD reduced at higher BSFL values, but it was significantly lower than the control ( $P < 0.001$ ) only at 100% BSFL inclusion. A similar pattern was observed for the FV, except for 20% BSFL inclusion which was not different from any other diet in the study ( $P < 0.05$ ). The control showed a significantly higher ( $P < 0.05$ ) SB compared to 80% BSFL inclusion, but no significant differences were observed among the other diets.

#### **Characteristics of kibbles**

The characteristics of the kibbles containing increasing levels of BSFL meal are presented in Table 3. Water activity and WAI increased with BSFL meal inclusion ( $P < 0.001$ ). As expected, WSI showed opposite pattern as it inversely proportional to WAI ( $P < 0.001$ ). Moreover, the increasing inclusion level of BSFL meal did not change the OAI. Regarding the CIELAB space parameters,  $L^*$  decreased with BSFL meal inclusion up to 80% ( $P < 0.001$ ), whereas  $a^*$  and  $b^*$  did not follow a consistent pattern. Nonetheless, noticeable changes in  $\Delta E$  were observed, with darker kibbles for 80 and 100% BSFL meal inclusion compared to the other diets. Over-

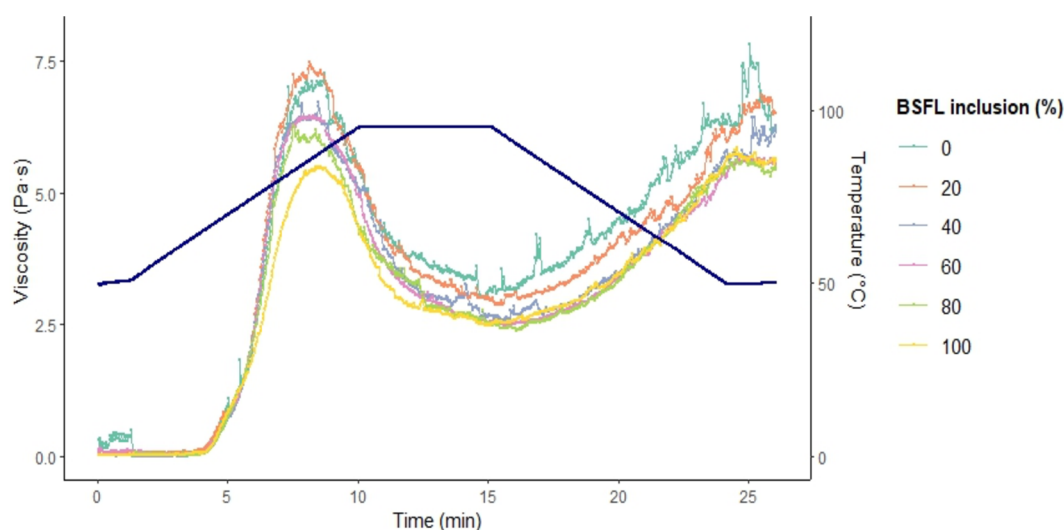


FIGURE 1 Pasting curves of the different experimental diets containing increasing levels of BSFL meal prior to extrusion.

TABLE 2 Pasting properties of the experimental diets containing increasing levels of BSFL meal prior to extrusion

Item	Control	Inclusion of BSFL meal					SEM	P value
		20%	40%	60%	80%	100%		
Pasting temperature (°C)	62.15	62.54	61.51	62.22	62.83	62.42	0.80	0.909
Peak viscosity (Pa·s)	7.20 <sup>a</sup>	7.05 <sup>ab</sup>	6.84 <sup>ab</sup>	6.62 <sup>ab</sup>	6.43 <sup>b</sup>	5.45 <sup>c</sup>	0.25	<0.05
Holding strength viscosity (Pa·s)	3.02 <sup>a</sup>	2.93 <sup>a</sup>	2.63 <sup>b</sup>	2.54 <sup>b</sup>	2.53 <sup>b</sup>	2.50 <sup>b</sup>	0.06	<0.001
Breakdown viscosity (Pa·s)	4.13 <sup>a</sup>	4.03 <sup>a</sup>	4.10 <sup>a</sup>	4.03 <sup>a</sup>	3.90 <sup>a</sup>	2.92 <sup>b</sup>	0.07	<0.001
Final viscosity (Pa·s)	6.63 <sup>a</sup>	6.16 <sup>ab</sup>	5.80 <sup>b</sup>	5.60 <sup>b</sup>	5.50 <sup>b</sup>	5.63 <sup>b</sup>	0.15	<0.05
Setback viscosity (Pa·s)	3.60 <sup>a</sup>	3.34 <sup>ab</sup>	3.20 <sup>ab</sup>	3.12 <sup>ab</sup>	2.96 <sup>b</sup>	3.12 <sup>ab</sup>	0.13	<0.05

Averages in the same row followed by different superscript letters are statistically different by the Tukey test ( $P < 0.05$ ). SEM, pooled standard error of means.

all, the BSFL meal reduced EI for all diets compared to control, except for 60% inclusion ( $P < 0.001$ ). For hardness, from 40% BSFL meal inclusion onwards, increased hardness was observed compared to control ( $P < 0.001$ ). As for the *in vitro* digestibility, significant reduction of DM was caused by the BSFL meal only at 100% inclusion ( $P < 0.001$ ) compared to the other diets. The CP digestibility, however, was significantly lower than the control for the inclusion levels between 60 and 100% BSFL meal.

### Microstructure

Control showed an irregular and porous microstructure, with pores of varying sizes that were formed after exiting the die. However, along with the increasing levels of BSFL, kibble microstructure became smoother, more regular, and less porous. Similarly, the outer layers of the pores became thicker with higher BSFL values. Scanning electron micrographs of kibbles containing different concentrations of BSFL meal are shown in Figure 2.

### Fourier-transformed infrared spectroscopy (FT-IR)

Given the complexity of the formulations, multiple bands at different wavenumbers can be distinguished. FT-IR spectra of all the different kibbles with varying contents of BSFL meal after normalization and baseline correction can be seen in Figure 3.

## 4 Discussion

During extrusion cooking, the mixture of ingredients undergo through a combination of moisture, pressure, temperature and shear, that combined with residence time inside the extruder barrel, transforming the matrix (Tran *et al.*, 2008), giving the final shape and texture to the kibbles (Kaelle *et al.*, 2024). This transformation depends on the specific mechanical energy (SME) and specific thermal energy (STE), which compose the total energy applied to the dough during extrusion (Monti *et al.*, 2016). However, alterations of extrusion SME,

TABLE 3 Characteristics of the kibbles containing increasing levels of BSFL meal

Control	Inclusion of black soldier fly larvae meal					SEM	P value
	20%	40%	60%	80%	100%		
Water activity ( $a_w$ ), WSI, WAI and OAI							
$a_w$	0.52 <sup>c</sup>	0.54 <sup>c</sup>	0.59 <sup>b</sup>	0.62 <sup>b</sup>	0.68 <sup>a</sup>	0.68 <sup>a</sup>	0.01 <0.001
WSI (%)	22.15 <sup>a</sup>	22.73 <sup>a</sup>	21.71 <sup>a</sup>	17.04 <sup>b</sup>	12.45 <sup>c</sup>	15.25 <sup>bc</sup>	1.09 <0.001
WAI (g/g)	3.38 <sup>d</sup>	3.41 <sup>d</sup>	3.49 <sup>cd</sup>	3.90 <sup>b</sup>	3.65 <sup>c</sup>	4.220 <sup>a</sup>	0.04 <0.001
OAI (ml/g)	1.80	1.62	1.40	1.44	1.61	1.40	0.11 0.115
Expansion index (EI) and hardness							
EI	2.21 <sup>ab</sup>	2.11 <sup>bc</sup>	2.12 <sup>bc</sup>	2.28 <sup>a</sup>	2.08 <sup>c</sup>	2.02 <sup>c</sup>	0.03 <0.001
Hardness (kgf/cm <sup>2</sup> ) <sup>2</sup>	0.99 <sup>c</sup>	1.16 <sup>bc</sup>	1.32 <sup>ab</sup>	1.44 <sup>a</sup>	1.70 <sup>a</sup>	1.80 <sup>a</sup>	- <0.001
Colour and total colour change ( $\Delta E$ )							
$L^*$	65.03 <sup>a</sup>	63.16 <sup>b</sup>	61.06 <sup>c</sup>	56.77 <sup>d</sup>	53.87 <sup>e</sup>	55.13 <sup>e</sup>	0.33 <0.001
$a^*$	7.76 <sup>c</sup>	8.99 <sup>b</sup>	9.24 <sup>ab</sup>	9.45 <sup>ab</sup>	8.84 <sup>b</sup>	9.90 <sup>a</sup>	0.19 <0.001
$b^*$	26.80 <sup>c</sup>	31.26 <sup>a</sup>	29.74 <sup>ab</sup>	29.30 <sup>ab</sup>	26.48 <sup>c</sup>	28.42 <sup>bc</sup>	0.57 <0.001
$\Delta E$	-	5.011 <sup>c</sup>	5.90 <sup>c</sup>	8.87 <sup>b</sup>	11.33 <sup>a</sup>	11.70 <sup>a</sup>	0.40 <0.001
<i>In vitro</i> digestibility (%)							
Dry matter	88.41 <sup>a</sup>	88.05 <sup>a</sup>	88.5 <sup>a</sup>	88.84 <sup>a</sup>	88.87 <sup>a</sup>	85.00 <sup>b</sup>	0.38 <0.001
Crude protein	94.91 <sup>a</sup>	93.97 <sup>ab</sup>	93.10 <sup>ac</sup>	92.48 <sup>bc</sup>	91.83 <sup>c</sup>	89.38 <sup>d</sup>	0.29 <0.001

Averages in the same row followed by different superscript letters differ significantly by Tukey's test ( $P < 0.05$ ). SEM, pooled standard error of means. <sup>2</sup>differ significantly by Kruskal-Wallis ( $P < 0.05$ ).

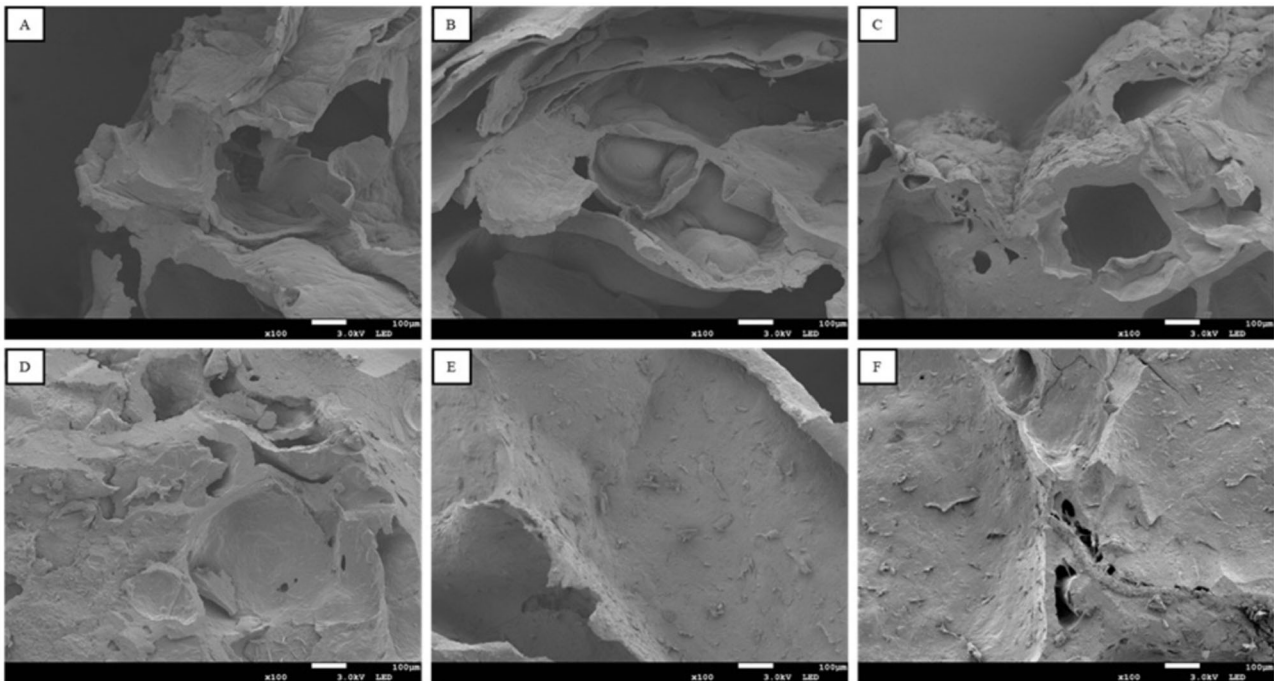


FIGURE 2 Scanning electron micrographs of kibbles containing increasing levels of black soldier fly (BSFL) meal. A, control; B, 20% BSFL; C, 40% BSFL; D, 60% BSFL; E, 80% BSFL; F, 100% BSFL.

and consequently STE, can radically affect starch gelatinization and kibble characteristics (Corsato Alvarenga *et al.*, 2021). It is known that the extrusion process is extremely sensitive to even the smallest variation in processing conditions (Le Guillas *et al.*, 2024) and chem-

ical composition of the formula (Tran *et al.*, 2008). In general, insoluble fibres increase the SME, leading to a lower starch gelatinization and a poor kibble characteristic (Monti *et al.*, 2016). This happens due to the hydration capacity of the insoluble fibres (Karkle *et al.*,

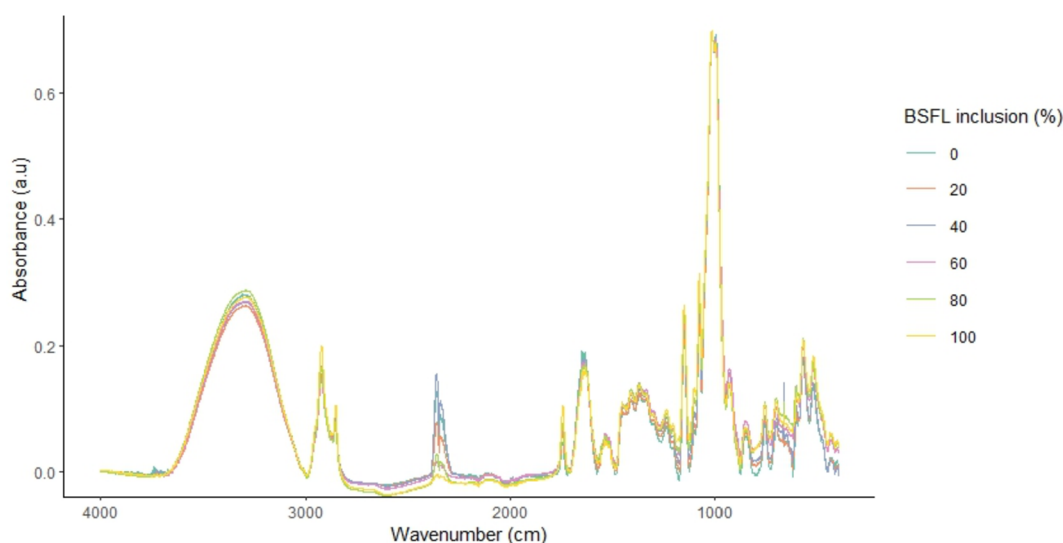


FIGURE 3 FT-IR spectra of the kibbles containing increasing levels of BSFL meal.

2012) retaining water, limiting its release for lubrication inside the barrel and starch gelatinization (BeMiller, 2011). Additionally, these molecules are unexpandable, which directly impacts the expandability of the kibbles (Pacheco *et al.*, 2021; Souza *et al.*, 2023). In our study, the increasing levels of BSFL meal resulted in higher crude fibre, which was expected due to the to the chitin, a highly insoluble cellulose-like compound naturally present in the insects (Abenaim and Conti, 2025). Also, increasing BSFL in dog food formulations also promotes higher SME (Chen *et al.*, 2025a). Thus, all the extruder settings and parameters were kept constant during the extrusion of all diets. Therefore, changes in kibble characteristics and *in vitro* digestibility can be directly related to the impacts of replacing poultry meal by increasing levels of BSFL meal.

After gelatinization, the pasting occurs under the conditions of continuous heating of starch granules in excessive water, leading to swollen granules that will be disrupted after polymer molecules release (BeMiller, 2011), resulting in increased viscosity. The PT is the temperature at which the viscosity of the starch starts to rise (Balet *et al.*, 2019). In this case, the BSFL inclusion did not affect PT, likely due to the similar starch content of the experimental diets. However, the decreased PV at 80 and 100% BSFL inclusion, may be explained by their higher levels of insoluble fibres, which may have absorbed part of the available water, limiting starch hydration and its swelling (Karkle *et al.*, 2012). Similarly, the high insoluble fibre levels led to a decreased HSV. The PV refers to starch gelatinization and indicates the maximum viscosity of the suspension before the disruption of the granules and leaching of the amylose (Corsato Alvarenga *et al.*, 2021). The HSV, in turn,

is associated to the dissolution of starch molecules, leading to melted crystalline regions and the BD, indicating integrity of the swollen granules of the gelatinized starch, disintegrated under shear (Balet *et al.*, 2019; BeMiller, 2011). Therefore, the increased insoluble fibre content caused by higher BSFL inclusion levels depleted starch hydration, integrity and melting, resulting in reduced BD and FV. Moreover, lower starch gelatinization leads to less leaching of amylose, reducing short-term retrogradation during cooling, which might explain, the significantly higher SB of the control compared to 80% BSFL inclusion. Similar results were reported for different inclusions of BSF and crickets in dog food (Chen *et al.*, 2025a). Nonetheless, the extrusion of the experimental diets was not compromised. This suggests that the changes in pasting properties are minor.

The natural characteristics of the insoluble fibres may lead to lower WSI, increased  $a_w$  and WAI (Balet *et al.*, 2019; BeMiller, 2011; Karkle *et al.*, 2011; Le Guillas *et al.*, 2024) and accordingly, these effects were observed for the increasing levels of BSFL meal. According to Igual *et al.* (2020), the WAI represents the amount of water immobilized by the extrudate, whilst the WSI is the amount of small molecules generated by molecular damage during the thermomechanical process. In their study, similar pattern was observed using increasing levels of crickets, supporting that high inclusion levels of insects lowers starch gelatinization during the extrusion process. Moreover, increasing levels of insoluble fibres, may also cause the reduction of EI and considerably higher hardness of the kibbles (Chen *et al.*, 2025b; Monti *et al.*, 2016). In fact, this pattern was observed with the increasing levels of BSFL and corroborate the findings

of Chen *et al.* (2025a) for increasing levels of BSFL in extruded dog food and Edah and Owolabi (2023) and Cheng *et al.* (2025) for BSFL-based extruded aquafeed. Even though the lowered EI, the findings of this study are satisfactory for formulations containing higher levels of insoluble fibre-like components. The findings of this research can be supported by those of Bill Kaelle *et al.* (2024), that reported similar results for high-density kibbles that were produced using starch sources with lower gelatinization.

Even though the increasing moisture levels, the  $a_w$  was higher only for the two highest BSFL inclusions. In closed systems, like extruders,  $a_w$  is influenced by type and composition of the matrix, physicochemical state and structure of the feed (Gautam *et al.*, 2020). The increase insoluble fibre, WAI and the lower gelatinization degree observed in this study may explain these differences as these factors reduce extensibility and induce residual vapor trap inside the structure of the extrudate (Cheng *et al.*, 2025b; Karkle *et al.*, 2012). Nonetheless, the  $a_w$  level is only considered hazardous when above 0.85 (Özgür and Yalçın, 2017). Therefore, the findings of this study are within safe limits, and microbial stability is not a concern.

The lowest *in vitro* DM digestibility observed at 100% BSFL can also be attributed to the chitin, which is a poorly digestible fraction that can lower DM digestibility in diets with high BSFL meal inclusion (Abenaim and Conti, 2025). Similarly, the decrease in CP *in vitro* digestibility based on the inclusion of BSFL may be explained by the increasing levels of chitin, which is a nitrogen-containing polysaccharide, and this could have overestimated the CP content in both, diets and residues (Penazzi *et al.*, 2021), as the crude protein analysis adopted in this study does not separate non-protein nitrogen. Similar results for BSFL-based dog food were reported by Penazzi *et al.* (2021) for DM and crude protein *in vitro* digestibility. Despite DM and CP reduced *in vitro* digestibility, the minimum recommended levels for digestibility of DM and CP are 70% and 80%, respectively (FEDIAF, 2024). Therefore, the increasing levels of BSFL meal do not hinder overall *in vitro* digestibility.

The CIELAB space parameters indicate that the inclusion of BSFL meal darkens the kibbles, which is in line with literature (Alam *et al.*, 2019). On a three-level scale (Adekunte *et al.*, 2010), the  $\Delta E$  of the diets can all be classified as very distinct, meaning that the colour difference among diets can be noticed at glance. However, significant discrepancies are perceptible from the inclusion of 40% BSFL onwards. The  $\Delta E$  change may be related to the phenolic compounds in the insect cuti-

cle that can be oxidated, forming protein-polyphenol interactions that are further catalysed by phenoloxidase (Mshayisa *et al.*, 2022), generating the brown colour. Also, BSFL meal is rich in iron, that according to Janssen *et al.* (2019), interact with polyphenols and induce a dark colouring. The authors also reported that these reactions occur during the gridding, which support the argument that the dark colorization of BSFL is not linked to extrusion. Although the colour is not a concerning factor for the dogs, it has been shown that pet owners are less receptive of dark coloured kibbles when purchasing (Ai *et al.*, 2025). Therefore, the impact of the BSFL meal on the kibble colour may negatively influence the commercialization of BSFL-based products.

A high-density, low-expansion diet suggest kibbles with low porosity (Tiwari and Jha S., 2017). In fact, while the control diet showed an irregular and expanded porous microstructure with thin layers, the progressive inclusion of BSFL led to a smoother, regular, less porous kibbles with thicker outer layers. These results may also be consequence of the increasing chitin of the experimental diets (Monti *et al.*, 2016; Souza *et al.*, 2023) and can be related to the lower EI and higher hardness results at increasing BSFL levels.

Bands are linked to molecular conformations and associated to matrix components (Kumar *et al.*, 2018; Mshayisa *et al.*, 2022; Queiroz *et al.*, 2021; Robertson *et al.*, 2024). The region between  $950\text{ cm}^{-1}$  and  $1200\text{ cm}^{-1}$  is typically called the starch fingerprint. In all cases, the most intense bands were located at  $998\text{ cm}^{-1}$  (C–O bending) and  $1015\text{ cm}^{-1}$  (C–O–C bending). The band at  $1153\text{ cm}^{-1}$  is attributed to the C–O stretching. As the starch is the main component of dog food, similar bands intensity was observed for all formulations with no difference in the quantity. On the other hand, the broad band between  $3000\text{ cm}^{-1}$  and  $3500\text{ cm}^{-1}$  corresponds to the O–H stretching that can be attributed mainly to the water molecules as well as to other hydroxyl-containing molecules. According to Kumar *et al.* (2018) and Queiroz *et al.* (2021) this band overlaps with the N–H stretching of chitin. In this research, the higher the BSFL, higher was the intensity of this band and this could be due to both higher water and chitin contents at higher BSFL inclusions. Similarly, bands associated to lipids ( $1745\text{ cm}^{-1}$ , C=O stretching,  $2850\text{ cm}^{-1}$  and  $2922\text{ cm}^{-1}$ , asymmetric and symmetric stretching of  $-\text{CH}_2$  and  $-\text{CH}_3$ , respectively) notably increased their intensity at higher BSFL levels. Furthermore, the amide I ( $1600\text{--}1700\text{ cm}^{-1}$ , C=O and N–H stretching) and amide II ( $1535\text{ cm}^{-1}$ , N–H bending and C–N stretching) regions can be linked to the presence of proteins

and chitin, since both molecules have these functional groups in their molecular structures. Given the sharp similarity in protein content among diets, different band intensities could be addressed to varying chitin content among formulations. Particularly, amide III ( $1233\text{ cm}^{-1}$ , C–N stretching and N–H bending) (Queiroz *et al.*, 2021) became progressively more intense at higher BSFL inclusion levels, and this could be explained particularly by the higher chitin contents.

## 5 Conclusions

Based on the findings of this study and considering the technological and manufacturing aspects, the partially defatted black soldier fly larvae meal can be recommended regardless of its inclusion level as: (1) the observed unfavourable effects on pasting properties did not compromise the extrusion process; (2) The effect of the increased insoluble fibre as a consequence of the chitin was irrelevant from an *in vitro* digestibility perspective. Nonetheless, it is still needed to apply the findings of this research in further *in vivo* studies to expand knowledge to their impacts on palatability, *in vivo* digestibility and pet health.

## Acknowledgements

We would like to thank Marina Van Hecke for helping with the extrusion, and Benny Lewille for the support on the Cryo-SEM visualization as well as United Petfood Group and Protix for donating the ingredients for this study, The Victam Foundation for donating the gravimetric feeder used in this research, and Andre Skirtach's support on the Fourier-transformed infrared spectroscopy (FTIR) measurements.

## Conflict of interest

The authors declare no conflict of interest.

## Funding

This study was part of a project supported by the Research Foundation Flanders (FWO), Belgium (grant number S001922N).

## References

- Abd El-Wahab, A., Meyer, L., Kölln, M., Chuppava, B., Wilke, V., Visscher, C. and Kamphues, J., 2021. Insect larvae meal (*Hermetia illucens*) as a sustainable protein source of canine food and its impacts on nutrient digestibility and fecal quality. *Animals* 11: 2525. <https://doi.org/10.3390/ani11092525>
- Abenaim, L. and Conti, B., 2025. Harnessing chitin from edible insects for livestock nutrition. *Insects* 16: 799. <https://doi.org/10.3390/insects16080799>
- Adekunte, A.O., Tiwari, B.K., Cullen, P.J., Scannell, A.G.M. and O'Donnell, C.P., 2010. Effect of sonication on colour, ascorbic acid and yeast inactivation in tomato juice. *Food Chemistry* 122: 500-507. <https://doi.org/10.1016/j.foodchem.2010.01.026>
- Ai, C., Akaichi, F., Glenk, K., Revoredo-Giha, C. and Costa-Font, M.L., 2025. What drives pet food choices? A systematic literature review. *Animals* 15: 3235. <https://doi.org/10.3390/ani15223235>
- Alam, M.R., Scampicchio, M., Angeli, S. and Ferrentino, G., 2019. Effect of hot melt extrusion on physical and functional properties of insect based extruded products. *Journal of Food Engineering* 259: 44-51. <https://doi.org/10.1016/j.jfoodeng.2019.04.021>
- Balet, S., Guelpa, A., Fox, G. and Manley, M., 2019. Rapid visco analyser (RVA) as a tool for measuring starch-related physicochemical properties in cereals: a review. *Food Analytical Methods* 12: 2344-2360. <https://doi.org/10.1007/s12161-019-01581-w>
- Baller, M.A., Pacheco, P.D.G., Peres, F.M., Monti, M. and Carciofi, A.C., 2018. The effects of in-barrel moisture on extrusion parameters, kibble macrostructure, starch gelatinization, and palatability of a cat food. *Animal Feed Science and Technology* 246: 82-90. <https://doi.org/10.1016/j.anifeedsci.2018.10.003>
- Baller, M.A., Pacheco, P.D.G., Vitta-Takahashi, A., Putarov, T.C., Vasconcellos, R.S. and Carciofi, A.C., 2021. Effects of thermal energy on extrusion characteristics, digestibility and palatability of a dry pet food for cats. *Journal of Animal Physiology and Animal Nutrition* 105: 76-90. <https://doi.org/10.1111/jpn.13606>
- BeMiller, J.N., 2011. Pasting, paste, and gel properties of starch-hydrocolloid combinations. *Carbohydrate Polymers* 86: 386-423. <https://doi.org/10.1016/j.carbpol.2011.05.064>
- Biagi, G., Cipollini, I., Grandi, M., Pinna, C., Vecchiato, C.G. and Zaghini, G., 2016. A new *in vitro* method to evaluate digestibility of commercial diets for dogs. *Italian Journal of Animal Science* 15: 617-625. <https://doi.org/10.1080/1828051X.2016.1222242>

- Bolat, B., Ugur, A.E., Oztop, M.H. and Alpas, H., 2021. Effects of high hydrostatic pressure assisted degreasing on the technological properties of insect powders obtained from *Acheta domesticus* and *Tenebrio molitor*. *Journal of Food Engineering* 292: 110359. <https://doi.org/10.1016/j.jfoodeng.2020.110359>
- Bosch, G., Vervoort, J.J.M. and Hendriks, W.H., 2016. *In vitro* digestibility and fermentability of selected insects for dog foods. *Animal Feed Science and Technology* 221: 174-184. <https://doi.org/10.1016/j.anifeedsci.2016.08.018>
- Bosch, G., Zhang, S., Oonincx, D.G.A.B. and Hendriks, W.H., 2014. Protein quality of insects as potential ingredients for dog and cat foods. *Journal of Nutritional Science* 3: e29. <https://doi.org/10.1017/jns.2014.23>
- Chen, Y., Graff, T., Cairns, A.C., Griffin, R., Silveru, K., Pezali, J.G. and Alavi, S., 2025a. Use of insect meals in dry expanded dog food: impact of composition and particulate flow characteristics on extrusion process and kibble properties. *Processes* 13: 2083. <https://doi.org/10.3390/pr13072083>
- Chen, Y., Weiss, T., Wang, D., Alavi, S. and Aldrich, C.G., 2025b. Optimizing *Aspergillus oryzae* inoculation dosage and fermentation duration for enhanced protein content in soybean meal and its influence on dog food extrusion. *Processes* 13: 2441. <https://doi.org/10.3390/pr13082441>
- Cheng, H., Thorsteinsdottir, A., Dalsgaard, T.K., Danielsen, M., Zatti, K.M. and Feyissa, A.H., 2025. Evaluation of the physical and chemical quality of Atlantic salmon feed with inclusion of full fat black soldier fly or mealworm meal: extrusion trials and modelling. *Animal Feed Science and Technology* 320: 116201. <https://doi.org/10.1016/j.anifeedsci.2024.116201>
- Corsato Alvarenga, I., Keller, L.C., Waldy, C. and Aldrich, C.G., 2021. Extrusion processing modifications of a dog kibble at large scale alter levels of starch available to animal enzymatic digestion. *Foods* 10: 2526. <https://doi.org/10.3390/foods10112526>
- D'Hondt, E., Soetemans, L., Bastiaens, L., Maesen, M., Jespers, V., Van den Bosch, B., Voorspoels, S. and Elst, K., 2020. Simplified determination of the content and average degree of acetylation of chitin in crude black soldier fly larvae samples. *Carbohydrate Research* 488: 107899. <https://doi.org/10.1016/j.carres.2019.107899>
- Donadelli, R.A., Dogan, H. and Aldrich, G., 2021. The effects of fiber source on extrusion parameters and kibble structure of dry dog foods. *Animal Feed Science and Technology* 274: 114884. <https://doi.org/10.1016/j.anifeedsci.2021.114884>
- Edah, B. and Owolabi, O.D., 2023. Physical properties of defatted and extruded black soldier fly (*Hermetia illucens*) larvae-based aqua-feed using a twin-screw extruder. *Discover Food* 3: 14. <https://doi.org/10.1007/s44187-023-00056-6>
- FEDIAF, 2024. Nutritional guidelines for complete and complementary pet food for dogs and cats. European Pet Food Industry Federation, Brussels.
- FEDIAF, 2025. Facts and figures. European Pet Food Industry Federation, Brussels.
- Finke, M.D., 2007. Estimate of chitin in raw whole insects. *Zoo Biology* 26: 105-115. <https://doi.org/10.1002/zoo.20123>
- Gautam, B., Govindan, B.N., Gänzle, M. and Roopesh, M.S., 2020. Influence of water activity on the heat resistance of *Salmonella enterica* in selected low-moisture foods. *International Journal of Food Microbiology* 334: 108813. <https://doi.org/10.1016/j.ijfoodmicro.2020.108813>
- Igual, M., García-Segovia, P. and Martínez-Monzó, J., 2020. Effect of *Acheta domesticus* (house cricket) addition on protein content, colour, texture, and extrusion parameters of extruded products. *Journal of Food Engineering* 282: 110032. <https://doi.org/10.1016/j.jfoodeng.2020.110032>
- Jafari, M., Koocheki, A. and Milani, E., 2017. Effect of extrusion cooking on chemical structure, morphology, crystallinity and thermal properties of sorghum flour extrudates. *Journal of Cereal Science* 75: 324-331. <https://doi.org/10.1016/j.jcs.2017.05.005>
- Janssen, R.H., Canelli, G., Sanders, M.G., Bakx, E.J., Lakemond, C.M.M., Fogliano, V. and Vincken, J.-P., 2019. Iron-polyphenol complexes cause blackening upon grinding *Hermetia illucens* (black soldier fly) larvae. *Scientific Reports* 9: 2967. <https://doi.org/10.1038/s41598-019-38923-x>
- Jian, S., Zhang, L., Ding, N., Yang, K., Xin, Z., Hu, M., Zhou, Z., Zhao, Z., Deng, B. and Deng, J., 2022. Effects of black soldier fly larvae as protein or fat sources on apparent nutrient digestibility, fecal microbiota, and metabolic profiles in beagle dogs. *Frontiers in Microbiology* 13: 1044986. <https://doi.org/10.3389/fmicb.2022.1044986>
- Kaelle, G.C.B., Bastos, T.S., Souza, R.B.M.D.S.D., Fernandes, E.L., Santos, L.N.A., Oliveira, S.G.D. and Félix, A.P., 2024. Starch sources and their influence on extrusion parameters, kibble characteristics and palatability of dog diets. *Italian Journal of Animal Science* 23: 388-396. <https://doi.org/10.1080/1828051X.2024.2313084>
- Karkle, E.L., Keller, L., Dogan, H. and Alavi, S., 2012. Matrix transformation in fiber-added extruded products: Impact of different hydration regimens on texture, microstructure and digestibility. *Journal of Food Engineering* 108: 171-182. <https://doi.org/10.1016/j.jfoodeng.2011.06.020>
- Keçińska-Pacelik, J. and Biel, W., 2022. Insects in pet food industry – hope or threat? *Animals* 12: 1515. <https://doi.org/10.3390/ani12121515>

- Kröger, S., Heide, C. and Zentek, J., 2020. Evaluation of an extruded diet for adult dogs containing larvae meal from the Black soldier fly (*Hermetia illucens*). *Animal Feed Science and Technology* 270: 114699. <https://doi.org/10.1016/j.anifeedsci.2020.114699>
- Kumar, R., Xavier, K.A.M., Lekshmi, M., Balange, A. and Gudipati, V., 2018. Fortification of extruded snacks with chitosan: Effects on techno functional and sensory quality. *Carbohydrate Polymers* 194: 267-273. <https://doi.org/10.1016/j.carbpol.2018.04.050>
- Le Guillas, G., Vanacker, P., Salles, C. and Labouré, H., 2024. Insights to study, understand and manage extruded dry pet food palatability. *Animals* 14: 1095. <https://doi.org/10.3390/ani14071095>
- Lei, X.J., Kim, T.H., Park, J.H. and Kim, I.H., 2019. Evaluation of supplementation of Defatted Black Soldier Fly (*Hermetia illucens*) larvae meal in Beagle dogs. *Annals of Animal Science* 19: 767-777. <https://doi.org/10.2478/aoas-2019-0021>
- Liadakis, G.N., Floridis, A., Tzia, C. and Oreopoulou, V., 1993. Protein isolates with reduced gossypol content from screw-pressed cottonseed meal. *Journal of Agricultural and Food Chemistry* 41: 918-922. <https://doi.org/10.1021/jf00030a016>
- Longvah, T., Mangthya, K. and Ramulu, P., 2011. Nutrient composition and protein quality evaluation of eri silkworm (*Samia ricinii*) prepupae and pupae. *Food Chemistry* 128: 400-403. <https://doi.org/10.1016/j.foodchem.2011.03.041>
- Loureiro, B.A., Oliveira, M.C.C., Peixoto, M.C., Ribeiro, E.M., Schauf, S., Castrillo, C. and Carciofi, A.C., 2024. Starch gelatinization implications for nutrient digestibility and fermentation products in the faeces of Beagle dogs. *Animal Feed Science and Technology* 309: 115894. <https://doi.org/10.1016/j.anifeedsci.2024.115894>
- Makkar, H.P.S., Tran, G., Heuzé, V. and Ankers, P., 2014. State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology* 197: 1-33. <https://doi.org/10.1016/j.anifeedsci.2014.07.008>
- Manbeck, A.E., Aldrich, C.G., Alavi, S., Zhou, T. and Donadelli, R.A., 2017. The effect of gelatin inclusion in high protein extruded pet food on kibble physical properties. *Animal Feed Science and Technology* 232: 91-101. <https://doi.org/10.1016/j.anifeedsci.2017.08.010>
- Monti, M., Gibson, M., Loureiro, B.A., Sá, F.C., Putarov, T.C., Villaverde, C., Alavi, S. and Carciofi, A.C., 2016. Influence of dietary fiber on macrostructure and processing traits of extruded dog foods. *Animal Feed Science and Technology* 220: 93-102. <https://doi.org/10.1016/j.anifeedsci.2016.07.009>
- Mshayisa, V.V., Van Wyk, J. and Zozo, B., 2022. Nutritional, techno-functional and structural properties of black soldier fly (*Hermetia illucens*) larvae flours and protein concentrates. *Foods* 11: 724. <https://doi.org/10.3390/foods11050724>
- Özgül, Y.B., 2017. Determination of some quality characteristics in pet foods. *Ankara Üniversitesi Veteriner Fakültesi Dergisi* 64: 21-24. [https://doi.org/10.1501/Vetfak\\_00000002768](https://doi.org/10.1501/Vetfak_00000002768)
- Pacheco, P.D.G., Baller, M.A., Peres, F.M., Ribeiro, É.D.M., Putarov, T.C. and Carciofi, A.C., 2021. Citrus pulp and orange fiber as dietary fiber sources for dogs. *Animal Feed Science and Technology* 282: 115123. <https://doi.org/10.1016/j.anifeedsci.2021.115123>
- Penazzi, L., Schiavone, A., Russo, N., Nery, J., Valle, E., Madrid, J., Martinez, S., Hernandez, F., Pagani, E., Ala, U. and Prola, L., 2021. *In vivo* and *in vitro* digestibility of an extruded complete dog food containing black soldier fly (*Hermetia illucens*) larvae meal as protein source. *Frontiers in Veterinary Science* 8: 653411. <https://doi.org/10.3389/fvets.2021.653411>
- Queiroz, L.S., Regnard, M., Jessen, F., Mohammadifar, M.A., Sloth, J.J., Petersen, H.O., Ajallouei, F., Brouzes, C.M.C., Fraihi, W., Fallquist, H., de Carvalho, A.F. and Casanova, F., 2021. Physico-chemical and colloidal properties of protein extracted from black soldier fly (*Hermetia illucens*) larvae. *International Journal of Biological Macromolecules* 186: 714-723. <https://doi.org/10.1016/j.ijbiomac.2021.07.081>
- Robertson, K., Ortuño, J., Stratakos, A., Stergiadis, S. and Theodoridou, K., 2024. Attenuated-total-reflection Fourier-transformed spectroscopy as a rapid tool to reveal the molecular structure of insect powders as ingredients for animal feeds. *Journal of Insects as Food and Feed* 10: 2143-2156. <https://doi.org/10.1163/23524588-00001092>
- Rolandelli, G., García-Navarro, Y.T., García-Pinilla, S., Farroni, A.E., Gutiérrez-López, G.F. and Buera, M.D.P., 2020. Microstructural characteristics and physical properties of corn-based extrudates affected by the addition of millet, sorghum, quinoa and canary seed flour. *Food Structure* 25: 100140. <https://doi.org/10.1016/j.foostr.2020.100140>
- Sogari, G., Amato, M., Biasato, I., Chiesa, S. and Gasco, L., 2019. The potential role of insects as feed: a multi-perspective review. *Animals* 9: 119. <https://doi.org/10.3390/ani9040119>
- Souza, C.M.M., Bastos, T.S., Kaelle, G.C.B., Bortolo, M., De Oliveira, S.G. and Félix, A.P., 2023. Fine cassava fibre utilization as a dietary fibre source for dogs: Effects on kibble characteristics, diet digestibility and palatability, faecal metabolites and microbiota. *Journal of Animal Physiology and Animal Nutrition* 107: 18-29. <https://doi.org/10.1111/jpn.13812>
- Tiwari, A. and Jha, S.K., 2017. Extrusion cooking technology: principal mechanism and effect on direct expanded snacks – an overview. *International Journal of Food Studies* 6: 113-128. <https://doi.org/10.7455/ijfs/6.1.2017.a10>

- Tran, Q.D., Hendriks, W.H. and van der Poel, A.F., 2008. Effects of extrusion processing on nutrients in dry pet food. *Journal of the Science of Food and Agriculture* 88: 1487-1493. <https://doi.org/10.1002/jsfa.3247>
- Valdés, F., Villanueva, V., Durán, E., Campos, F., Avendaño, C., Sánchez, M., Domínguez-Araujo, C. and Valenzuela, C., 2022. Insects as feed for companion and exotic pets: a current trend. *Animals* 12: 1450. <https://doi.org/10.3390/ani12111450>
- Wanapat, M., Cherdthong, A., Phesatcha, K. and Kang, S., 2015. Dietary sources and their effects on animal production and environmental sustainability. *Animal Nutrition* 1: 96-103. <https://doi.org/10.1016/j.aninu.2015.07.004>
- Wu, J., Xu, S., Yan, X., Zhang, X., Xu, X., Li, Q., Ye, J. and Liu, C., 2022. Effect of homogenization modified rice protein on the pasting properties of rice starch. *Foods* 11: 1601. <https://doi.org/10.3390/foods11111601>