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RESEARCH ARTICLE

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Bioconversion of expired canned cat food via Black Soldier Fly larvae: a sustainable approach to waste valorisation

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ABSTRACT

The pet food industry generates significant waste, including expired products that are often discarded, contributing to environmental concerns. This study explores the potential of using expired canned cat food as a rearing substrate for Black Soldier Fly Larvae (BSFL, *Hermetia illucens*), evaluating its impact on larval growth and nutrient composition. Three expired canned cat foods (beef, chicken and salmon-based) were tested against a commercial broiler diet as a control (CTRL). Results indicate that BSFL successfully developed on all tested substrates, with the salmon-based diet (SD) yielding the highest growth rate (0.010 g/day, $p < 0.01$) and nutrient assimilation, similar to the control group. The beef-based diet (BD) resulted in lower lipid accumulation (16.98% DM, $p < 0.05$), while all substrates influenced the larvae's protein (ranging from 43.12% to 45.64% DM) and fatty acid profiles. Notably, lauric acid, a key antimicrobial and metabolic compound, remained predominant in all larvae, with values ranging from 42.18% (BD) to 60.34% (CTRL) of total fatty acids ($p < 0.01$). These findings highlight the feasibility of expired pet food as a valuable resource for insect farming, promoting circular economic principles. However, regulatory constraints currently limit their use in feed applications. Future research should address legal considerations and optimise substrate formulations to enhance larval performance and industrial applications.

HIGHLIGHTS

- Expired cat food can be used as a substrate for Hermetia illucens reduces waste.
- Larvae grown on different substrates highlighted the influence of diet on their nutritional quality.
- The salmon-based diet promoted faster growth and better conversion efficiency.

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Pet food; *Hermetia illucens*; growth performance; chemical-nutritional characteristics of larvae

Introduction

In Europe the global population of pets is estimated to be 352 million animals with 30.1% of dogs and 36.6% of cats (Fediaf 2024). The annual sales of pet food products are estimated to be around 9.9 million tons, with an average value growth rate of the pet food industry in the last 3 years of 5.1% (Fediaf 2024). According to the annual report of ASSALCO (2024), in Italy, a population of 8.8 million of dogs and 10.2 million of cats was estimated in 2023, and, in the same year, the market of dog and cat food developed a turnover of 3,007 million euros for a total of 673,153 tons sold, with further growth prospects for the coming years. With a view to global market growth, it is reasonable to expect an increase in the waste from this industry, also in the form of expired products. To the best of our knowledge, no data is available in the literature regarding the quantity of the pet food discarded because it has reached or exceeded the 'best before date' shown on the packaging. However, these quantities are likely to be substantially high and consequently a considerable amount of waste. In fact, while there is not specific data on the amount of expired pet food wasted in Europe, it is clear that food waste represents a significant issue

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(European Environment Agency 2025), and pet food is likely included in those figures. The European Commission estimates that the EU are wastes are 88 to 129 million tons of food annually, with associated costs of 143 billion euros. Pet food, like other food products, has a shelf life and can be discarded if it expires or if its quality deteriorates. In addition, according to Fediaf (2024), the annual volume growth rate for the pet food industry is estimated as 2% for the next years. The disposal of expired pet food can be a problem for both manufactures and retail stores (Xu and Li 2012). According to the European indications, the date of minimum durability ('best before') reported in the food packages indicates the end of the period under any stated storage conditions, during which the product will remain fully marketable and will retain any specific attributes for which tacit or express claims have been made (Soethoudt et al. 2013). However, beyond the 'best before' date, the food may still be perfectly satisfactory from a nutritional and safety point of view. A possible re-utilisation and valorisation of these wastes, particularly rich in protein and other macro- and micro-nutrients, could be their use as a growing substrate for bioconverter insect larvae, such as *Hermetia illucens* (also known as Black Soldier Fly, BSF). BSF is one of the most studied insects for mass rearing due to its ability to efficiently convert organic waste into high-protein biomass while simultaneously reducing environmental impact (Addeo et al. 2022; Scieuzo et al. 2023). Recent studies highlighted the safety of using organic waste as insect substrates (Opoku et al. 2023). BSF is a powerful bio-converting insect, able to use a wide range of substrates to produce a larval biomass rich not only in protein and lipids, but also in bioactive compounds such as chitin, lauric acid and antimicrobial peptides (Franco et al. 2021; Scieuzo et al. 2023; Addeo et al. 2024; Franco et al. 2024) with possible uses in animal nutrition but also in different kinds of industry (Franco et al. 2021; Lo et al. 2024). In order to contribute to the sustainability of the pet food industry, and to explore new potential substrates for BSF growth, the aim of our research was to use wet cat food discarded from the market after the 'best before' date as a substrate for BSF Larvae (BSFL) growth. The considered wet food was chosen because it has approximately 78% moisture, an optimal value for the development of BSFL (Okpoko et al. 2024; Nayak and Klüber, 2025). Our hypothesis was that an expired substrate, originally formulated to fully meet the nutritional requirements of a monogastric animal, could serve as a suitable nourishment source for BSFL.

Materials and methods

Preparation for BSFL diets

A total of four substrates were used in the trial. A commercial broiler diet (starter diet, Veronesi S.p.A., Verona, Italy) was used for the control group (CTRL). The other three substrates were represented by three complete canned food for adult cats named beef, chicken and salmon mousses. The cat foods were provided by a reputable pet food manufacturer (Farmina, Nola, Italy) and, at the time of use, had expired between one and three months. For each type of product beef diet (BD), chicken diet (CD) and salmon diet (SD), three packages were chosen at random from a higher amount of different canned diets, and the moisture content was determined; for all types, the percentage of moisture was 77.4, 78.1 and 79.0%, with a correspondent dry matter content of 22.6, 21.9 and 21.0%, respectively for BD, CD and SD. After the moisture was measured, the cans were opened, and the water content of the substrates was reduced in a ventilated oven at low temperature (40 °C) for approximately 24 h, until it reached values close to 70%. Prior to this transfer, the cans had been stored at room temperature between 18 and 22 °C, in a cool dry environment, as directed by the manufacturer. When the cans of cat food were opened, no anomalous odours or colours indicating a possible alteration of the product were evident. A batch feeding treatment was applied for BSFL growth, meaning that the four substrates were placed in a plastic tank ($60 \times 40 \times 15$ cm) one day prior to introducing the larvae, at the beginning of the experiment. The substrates were left to preheat until the start of the experiment in a growth chamber at 27 °C, allowing for a natural rise in temperature before larval introduction. Each container held 2500 g of substrate, to ensure a height of 10 cm as indicated by Peng et al. (2022). Each group had six replicate tanks.

The chemical-nutritional characteristics of the substrates are reported in Table 1.

Bioconversion parameters

BSFL were supplied by the start-up X-flies (Potenza, Italy). Larvae were shipped by the company at five days of age, arrived at the facilities of the Department of Veterinary Medicine and Animal Production of the University of Napoli Federico II (Napoli, Italy) at six days old, and were placed on the substrates at seven days of age. During the first six days of life, BSFL were fed by a standard Gainesville diet (Hogsette 1992).

Table 1. Chemical characteristics and fatty acid profile of the substrates utilised as feed for BSFL growth.

Diet	Control	Beef	Chicken	Salmon
Moisture, %	70.05	69.95	70.15	70.33
Crude protein, % DM	22.69	14.65	15.61	16.67
Lipids, % DM	5.12	9.55	9.22	10.11
Crude fibre, % DM	4.32	0.70	0.78	0.72
Ashes, % DM	3.69	4.44	4.58	4.22
Nitrogen free extract, % DM	68.50	71.36	70.59	69.00
Gross energy (Kcal/kg)	4734	4841	4833	4903
Fatty acid profile, g FA/100 g total FAME				
C10:0	0.00	0.06	0.050	0.08
C12:0	0.23	0.35	0.14	0.55
C14:0	0.21	1.22	1.07	1.67
C14:1n-5	0.04	0.18	0.16	0.17
iso-C15:0	0.03	0.04	0.03	0.04
anteiso-C15:0	0.01	0.04	0.023	0.03
C15:0	0.11	0.16	0.13	0.17
iso-C16:0	0.00	0.02	0.01	0.01
C16:0	19.45	24.24	24.68	22.85
C16:1n-9	0.11	0.41	0.46	0.43
C16:1n-7	0.34	4.21	4.23	3.85
C17:0	0.15	0.26	0.21	0.26
C17:1	0.01	0.02	0.02	0.01
C18:0	2.68	9.44	9.74	9.35
C18:1n-9(cis + trans)	29.00	33.18	34.52	32.34
C18:1n-7	1.29	2.11	2.17	2.17
C18:2n-6	40.11	18.30	16.36	16.40
C18:3n-6	0.03	0.10	0.12	0.10
C18:3n-4	N.D.	0.03	0.03	0.06
C18:3n-3	1.50	2.17	2.02	2.33
C18:4n-3	N.D.	0.03	0.06	0.11
C20:0	0.46	0.11	0.11	0.15
C20:1n-11	0.09	0.06	0.06	0.11
C20:1n-9	0.82	0.37	0.44	0.90
C20:1n-7	N.D.	0.01	0.03	0.03
C20:2n-6	0.18	0.15	0.13	0.22
C20:3n-6	N.D.	0.31	0.16	0.17
C20:4n-6	0.01	1.18	1.72	2.16
C20:3n-3	0.02	0.04	0.04	0.10
C20:4n-3	0.02	0.10	0.02	0.12
C20:5n-3	0.03	0.22	0.16	0.54
C22:0	0.32	0.05	0.04	0.06
C22:1n-11	0.86	0.01	0.04	0.53
C22:1n-9	1.10	0.03	0.05	0.11
C22:4n-6	N.D.	0.15	0.14	0.19
C22:5n-6	0.01	0.03	0.03	0.07
C22:5n-3	N.D.	0.34	0.20	0.31
C24:0	0.41	0.07	0.07	0.08
C22:6n-3	N.D.	0.17	0.28	1.09
C24:1n-9	N.D.	0.04	0.06	0.12
SFA	24.12	36.05	36.30	35.28
MUFA	33.66	40.63	42.24	40.76
n-6 PUFA	40.40	20.21	18.66	19.30
n-3 PUFA	1.57	3.00	2.77	4.59
n-6/n-3	25.73	6.58	6.74	4.20

Abbreviations: BSFL, Black Soldier Fly Larvae; FAME, fatty acid methyl esters; N.D., not determined; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

Beef: Chicken, beef (5%), herring, chicken liver, hydrolysed animal protein, rice, vitamins, minerals. Nutritional additives: Vitamin A 4000 IU, Vitamin D 400 IU, Vitamin E 130 mg, Taurine 800 mg/kg. *Chicken*: Chicken, herring, chicken liver, hydrolysed animal protein, rice, vitamins, minerals. Nutritional additives: Vitamin A 4000 IU, Vitamin D 400 IU, Vitamin E 130 mg, Taurine 800 mg/kg. Gross Energy (Kcal/kg) = (5.7 x % of crude proteins) + (9.4 x % lipids) + 4.1 x (% of carbohydrates + % of fibers) − 2. *Salmon*: Chicken, salmon (5%), herring, chicken liver, hydrolysed fish protein, rice, vitamins, minerals. Nutritional additives: Vitamin A 4000 IU, Vitamin D 400 IU, Vitamin E 130 mg, Taurine 800 mg/kg.

At seven days of age, BSFL were weighed in batches of 100 individuals and assigned to different dietary treatments to have a similar average larval weight/tank (0.174 ± 0.016 g corresponding to a mean individual weight of approximately 0.002 g). A total of 1,200 larvae were introduced in each tank to have a feeding rate of 0.083 g/larvae per day. The feeding rate was estimated based on our previous internal trials and to cover a growing period of about 25 days. The tanks were covered with a perforated cap with a black nylon grid and placed in a ventilated chamber (air flow around 2 m/s) under controlled environmental conditions (T: 27 ± 0.5 °C; RH: 70 ± 5%). On days 10, 12, 14, 16, 20 and 22 of age, one hundred larvae per replicate and per diet were randomly selected, weighed, and measured for length and thickness (measured at the middle of each larva) and were then returned to their respective tanks. Body Shape Index (BSI) of BSFL was determined to evaluate morphological variations in response to different food substrates. The BSI was calculated to assess larval morphology throughout development. This index provides a quantitative measure of body elongation, with higher values indicating a slenderer shape and lower values corresponding to a shorter, wider form. Since BSI reflects morphological changes that occur as larvae grow and prepare for the prepupal stage, it serves as a useful parameter to monitor developmental progression and potential influences of dietary substrates (Bonelli et al. 2020; Russell and Cator 2022; Eriksen 2024). The calculation was carried out by applying the following formula:

$$\text{Body Shape Index (BSI)} = (\text{body thickness}/\text{body length}) \text{ x } 100$$

Feeding BSFL continued until more than 25% of the larvae in a tray had developed into prepupae. That happens on day 20 of age for CTRL and SD groups and on day 22 for age BD and CD groups. The evaluation of the prepupae percentage has been undertaken by collecting exactly 100 g of substrate + larvae from each container and counting the number of larvae and prepupae contained in each replicate.

In addition, the following parameters were measured:

$$\text{Larval mortality (LM)}, \% = (\text{ILN} - (\text{FLN} + \text{FPN})) \times 100/\text{ILN}$$
$$\text{Growth rate (GR)}, \% = (\text{LFW} - \text{LIW})/\text{d}$$
$$\text{Reduction index (RI)}, \% = (\text{AS} - \text{RS}) \times 100/\text{AS}$$
$$\text{Bioconversion Efficiency Residue(BER)}, \% = (\text{Larval dry mass}/\text{dry mass of feed provided}) \times 100$$
$$\text{Waste reduction index (WRI)}, \% = (\text{AS} - \text{RS}) \times 100/\text{AS}/\text{d}$$

where ILN = initial larval number; FLN = final larval number; FPN = final prepupae number; FLW = final

larval weight; ILW = initial larval weight; d = days of the trial; AS = administered substrate; RS = residual substrate.

All the weights used for the calculation of performance indices are expressed on a dry matter basis. The bioconversion efficiency rate was calculated as proposed by Bosch et al. (2020).

At the end of the test, larvae were separated from the substrates by manual sieving, initially using a 1 × 4 mm mesh sieve (1 mm bar thickness and 4 mm aperture) to remove the larger particles. Subsequently, after a 24–hour fasting period in an empty tank to allow the digestive tract to empty, a second 1 × 2 mm mesh sieve was used to separate the larvae from the finer particles. Then, the larvae were freeze-dried using a Micromudulyo freeze dryer (Thermo Electron Corporation, Thermo Fisher Scientific Inc., Waltham, MA, USA) and their chemical composition was analysed. Dry matter (DM), ashes, and crude protein (CP) were analysed according to AOAC (2002). In brief, for DM and ashes, around 2.5 g of sample were weighed into a porcelain capsule and put in an electric oven at 103 °C until constant weight; then, the capsule was transferred to an electric stove at 550 °C for the whole night. The crude protein was determined using the Kjeldahl method; only for larvae, the nitrogen to crude protein conversion ratio utilised was 4.76 according to Jansen et al. (2017). Total lipids were extracted from each sample according to the method by Folch et al. (1957) and then gravimetrically quantified. The amount of nitrogen free extract (NFE) in the diets was calculated as follows: NFE, % DM = 100—Ashes, % DM—CP, % DM—Lipids, % DM. Neutral and polar lipids, (NL and PL, respectively) were separated following the method proposed by Juaneda and Rocquelin (1985), using Sep-Pak Silica column (Waters, Milford, MA, USA). NL were eluted using 20 mL of chloroform, while the PL with 30 mL of methanol. After solvent evaporation, lipid fractions were analysed for their fatty acids (FAs) composition using the same procedure applied to the total lipids. In brief, all the lipid extracts (i.e. total lipids, NL and PL) were transesterified to methyl esters (FAME) (Christie 1982) and their fatty acid (FA) composition was determined using a Varian GC gas chromatograph (Varian Inc., Palo Alto, CA, USA). FAs were identified and then quantified by calibration curves by using tricosanoic acid (C23:0; 0.4 mg/mL) (Supelco, Bellefonte, PA, USA) as internal standard. Finally, cholesterol contained in the larvae was determined by gas chromatography analysis, according to Secci et al. (2018).

Statistical analysis

Data were analysed using one-way ANOVA with the GLM procedure of SAS (2002), according to the model: $Yij = \mu + Si + eij$, where Y is the single observation, μ the overall mean, S the effect of the substrate (i = CTRL, BD, CD, or DS), and e the experimental error. The normality of residuals was assessed using the Shapiro-Wilk test; all analysed data showed a distribution consistent with normality ($p > 0.05$), justifying the use of ANOVA. Homogeneity of variances was evaluated using Levene's test, which confirmed equal variances among groups ($p > 0.05$). Mean comparisons were performed using the Tukey HSD test, chosen for its ability to control type I error in multiple comparisons. Significance levels were set at $p < 0.05$ (significant) and $p < 0.01$ (highly significant).

Results

Table 2 presents the live weight of BSFL across different growing substrates. No differences were observed among groups until day 12. At days 14 and 16, larvae from the SD group had the highest live weight ($p < 0.01$). By day 20, larvae from CTRL and SD groups had similar weights, both higher ($p < 0.01$) than those in BD and CD groups. At day 22, larvae reared on CD substrate had a higher live weight ($p < 0.05$) than those in BD group. Body length of the larvae according to the growing substrate is reported in Table 3. Starting from 14 days of age, differences ($p < 0.01$) appeared among the groups. In particular, at day 14 SD and CTRL group showed a higher larval length than the larvae of BD and CD groups, while on day 20 the larvae of CD group had a higher larval length than those of CTRL and BD groups. On day 22, the larval length of the CD group was higher ($p < 0.01$) than that of the BD group. The body thickness of the larvae (Table 4) on day 16 was higher ($p < 0.05$) in SD than in BD group. On day 20 the SD group showed larvae with a body thickness higher ($p < 0.01$) than that of the CTRL and BD groups, while at 22 days no differences were recorded between the two remaining groups

Table 2. Live weight (g) of BSFL along the trial.

Group	Control	Beef	Chicken	Salmon	RMSE	*P*-value
D7	0.085	0.086	0.087	0.086	0.006	0.8254
D10	0.143	0.146	0.141	0.143	0.01	0.7329
D12	0.173	0.175	0.175	0.173	0.017	0.9852
D14	0.197^{B}	0.185^{B}	0.199^{B}	0.220^{A}	0.015	<.0001
D16	0.226^{B}	0.207^{C}	0.226^{B}	0.250^{A}	0.013	<.0001
D20	0.253^{A}	0.225^{B}	0.229^{B}	0.267^{A}	0.017	<.0001
D22	–	0.227^{b}	0.245^{a}	–	0.018	0.0334

A, B: $p < 0.01$; a, b: $p < 0.05$;
Abbreviations: BSFL, Black Soldier Fly Larvae; D, Day; RMSE, root mean square error.

Table 3. Body length (cm) of BSFL along the trial.

Group	Control	Beef	Chicken	Salmon	RMSE	*P*-value
D7	1.26	1.25	1.26	1.25	0.09	0.8329
D10	1.49	1.43	1.43	1.52	0.10	0.6214
D12	1.71	1.65	1.64	1.70	0.106	0.4067
D14	1.94^{A}	1.74^{B}	1.80^{B}	1.94^{A}	0.095	<.0001
D16	1.94	1.93	1.98	1.96	0.086	0.5730
D20	1.93^{B}	1.96^{B}	2.06^{A}	2.00^{AB}	0.051	0.0002
D22	–	1.98^{B}	2.07^{A}	–	0.040	0.0002

A, B: $p < 0.01$.
Abbreviations: BSFL, Black Soldier Fly Larvae; D, Day; RMSE, root mean square error.

Table 4. Body thickness (cm) of BSFL along the trial.

Group	Control	Beef	Chicken	Salmon	RMSE	*P*-value
D7	0.39	0.39	0.38	0.39	0.01	0.6896
D10	0.42	0.41	0.42	0.43	0.03	0.2569
D12	0.44	0.44	0.43	0.44	0.015	0.1931
D14	0.45	0.44	0.47	0.47	0.019	0.0681
D16	0.48^{ab}	0.47^{b}	0.49^{ab}	0.50^{a}	0.020	0.0301
D20	0.49^{B}	0.49^{B}	0.50^{AB}	0.51^{A}	0.011	<.0001
D22	–	0.49	0.50	–	0.009	0.2289

A, B: $p < 0.01$; a, b: $p < 0.05$.
Abbreviations: BSFL, Black Soldier Fly Larvae; D, Day; RMSE, root mean square error.

Table 5. Body shape index (cm/cm) of BSFL along the trial.

Group	Control	Beef	Chicken	Salmon	RMSE	*P*-value
D7	30.95	31.20	30.16	31.21	0.325	0.6512
D10	28.19	28.67	29.37	28.29	0.278	0.7423
D12	25.02	26.46	26.02	25.68	2.06	0.8517
D14	23.26^{B}	25.43^{A}	26.01^{A}	24.72^{AB}	1.30	0.003
D16	24.74	24.50	24.97	25.38	1.12	0.3450
D20	25.01^{AB}	24.82^{AB}	24.28^{B}	25.71^{A}	0.77	0.0024
D22	–	24.67^{a}	23.84^{b}	–	0.728	0.0194

A, B: $p < 0.01$; a, b: $p < 0.05$.
Abbreviations: BSFL, Black Soldier Fly Larvae; D, Day; RMSE, root mean square error.

(BD and CD). Table 5 reports BSI of the BSFL. On day 14 BD and CD groups showed a higher value than the control; on day 20 the SD group had a higher ($p < 0.01$) BSI than the CD group and at day 22 BD group showed a higher value ($p < 0.05$) than the CD group.

Table 6 summarises the growth indexes of the BSFL according to the different growing substrates. SD group larvae had a higher ($p < 0.01$) growth rate than the BD and CD group larvae, and BD group larvae had a lower growth rate than CTRL and SD group larvae. CTRL group showed a mortality rate higher than the CD and the SD groups, while the BD group had a mortality rate higher than that of the SD group. The SRI was the highest ($p < 0.01$) in the SD and CD groups, followed by BD and then by CTRL group. The WRI reached the highest ($p < 0.01$) value in the CTRL group and the lowest in the BD group. The chemical-nutritional characteristics, the fatty acid profile of the total lipids and cholesterol content of the BSFL are presented in Table 7. SD substrate produced larvae with a crude protein content higher ($p < 0.05$) than

Table 6. Growth performance of BSFL at the end of the trial.

Group	Control	Beef	Chicken	Salmon	RMSE	*P*-value
Growth rate, g/d	0.009^{AB}	0.005^{C}	0.006^{BC}	0.010^{A}	0.003	<.0001
Mortality, %	7.78^{A}	7.18^{AB}	6.61^{BC}	6.25^{C}	0.730	0.0002
SRI	78.30^{C}	86.90^{B}	88.68^{A}	89.90^{A}	1.43	<.0001
WRI	8.70^{A}	7.90^{C}	8.03^{B}	8.17^{B}	0.13	<.0001

A, B, C: $p < 0.01$.
Abbreviations: BSFL, Black Soldier Fly Larvae; RMSE, root mean square error; SRI, substrate reduction index; WRI, waste reduction index.

Table 7. Proximate composition, cholesterol content and fatty acid profile of *Hermetia illucens* larvae.

Group	Control	Beef	Chicken	Salmon	RMSE	*P*-value
Moisture, %	66.33	68.25	65.67	66.80	4.78	0.2569
Crude protein, % DM	44.63^{ab}	43.12^{b}	44.29^{ab}	45.64^{a}	1.09	0.0112
Ashes (% on DM)	7.45^{B}	10.84^{A}	10.22^{A}	7.82^{B}	0.59	<.0001
Total lipids (% DM)	23.23^{a}	16.98^{b}	23.17^{a}	24.65^{a}	3.89	0.039
Cholesterol (mg/100 g DM)	0.00^{C}	56.02^{B}	58.97^{AB}	82.23^{A}	17.92	0.0001
Fatty acids profile, g/100 g total fatty acid methyl esters						
C12:0	60.34^{A}	42.18^{D}	47.63^{B}	45.23^{C}	1.51	<.0001
C14:0	9.05^{A}	6.36^{B}	6.93^{B}	6.51^{B}	0.19	<.0001
C16:0	8.31^{C}	15.20^{A}	14.13^{B}	13.88^{B}	0.47	<.0001
C16:1n-9	0.21^{D}	1.03^{C}	1.11^{B}	1.19^{A}	0.04	<.0001
C16:1n-7	2.18^{C}	5.27^{A}	4.54^{B}	4.40^{B}	0.14	<.0001
C18:0	1.19^{B}	1.68^{A}	1.28^{B}	1.58^{A}	0.12	<.0001
C18:1n-9	7.09^{C}	17.69^{A}	14.96^{B}	15.96^{B}	0.83	<.0001
C18:1n-7	0.60^{B}	1.32^{A}	0.72^{B}	1.19^{A}	0.21	0.0003
C18:2n-6	7.67^{A}	5.71^{B}	5.89^{B}	6.25^{B}	0.65	0.0024
C18:4n-3	1.44^{A}	0.83^{B}	0.61^{C}	0.73^{BC}	0.16	<.0001
SFA	79.71^{A}	66.05^{D}	70.40^{B}	67.79^{C}	0.90	<.0001
MUFA	10.44^{C}	25.69^{A}	21.67^{B}	23.13^{B}	1.17	<.0001
n-6 PUFA	7.72	6.60	6.62	6.84	0.70	0.1082
n-3 PUFA	2.00^{A}	1.42^{B}	1.21^{B}	2.10^{A}	0.21	<.0001
n-6/n-3	3.92^{C}	4.67^{B}	5.46^{A}	3.26^{D}	0.28	<0.0001

The following FAs (found in percentage below 1% of total FAME) were utilised for calculating the Σ classes of FAs but they are not listed in the table: C13:0, C14:1n-5, C15:0, anteisoC15:0, isoC15:0, isoC16:0, C16:2n-4, C17:0, C17:1, C18:2n-4, C18:3n-6, C18:3n-3, C18:4n-1, C20:0, C20:1n-11, C20:1n-7, C20:1n-9, C20:0, C20:4n-6, C20:5n-3, C21:5n-3, C22:0, C22:1n-11, C22:4n-6, C22:6n-3;
A, B, C: $p < 0.01$; a, b: $p < 0.05$.
Abbreviations: RMSE, root mean square error; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

that of larvae grown on BD substrate. BD and CD larvae had a higher ($p < 0.01$) ash content than CTRL and SD larvae and BD larvae had the lowest ($p < 0.05$) percentage of total lipids. Larvae grown on the SD substrate had a higher ($p < 0.05$) content of cholesterol than BD and CTRL group. In the latter group cholesterol was not detected. Lauric acid (C12:0) content was the highest ($p < 0.01$) in CTRL larvae, followed by CD (-21.0%), SD (-25.0%) and BD larvae (-30.0%). The same trend was observed for saturated fatty acids (SFA). BD larvae had a higher ($p < 0.01$) percentage of monounsaturated fatty acids (MUFA) than the other groups and the CTRL group had a lower MUFA content than the other groups. The content of n-3 polyunsaturated fatty acids (PUFA) of SD and CTRL larvae was higher ($p < 0.01$) than that of CD and BD groups. The n-6/n-3 ratio was the highest ($p < 0.01$) for CD larvae, followed by BD, CTRL and SD larvae. The fatty

Table 8. Fatty acids of the neutral lipid fraction (g/100 g total fatty acid methyl esters) of BSFL freeze-dried larvae.

Group	Control	Beef	Chicken	Salmon	RMSE	*P*-value
C10:0	1.45^{A}	1.09^{B}	1.13^{B}	1.20^{AB}	0.06	<.0001
C12:0	62.11^{A}	44.34^{C}	47.69^{B}	44.98^{C}	1.40	<.0001
C16:0	8.18^{C}	14.99^{A}	14.40^{A}	14.19^{B}	0.43	<.0001
C16:1n-9	0.19^{C}	1.00^{C}	1.08^{B}	1.17^{A}	0.05	<.0001
C16:1n-7	2.06^{C}	5.03^{A}	4.34^{B}	4.24^{B}	0.21	<.0001
C18:0	1.12^{B}	1.63^{A}	1.28^{B}	1.65^{A}	0.11	<.0001
C18:1n-9	6.33^{C}	16.31^{A}	14.25^{B}	15.31^{AB}	0.91	<.0001
C18:1n-7	0.56^{B}	1.25^{A}	0.70^{B}	1.17^{A}	0.20	0.0003
C18:2n-6	6.87^{a}	5.29^{b}	5.55^{b}	5.96^{ab}	0.67	0.0192
SFA	82.90^{A}	69.21^{C}	71.99^{B}	69.25^{c}	1.11	<.0001
MUFA	9.51^{C}	23.89^{A}	20.69^{B}	22.23^{ab}	1.27	<.0001
n-6 PUFA	6.97	6.28	6.69	6.88	0.60	0.3476
n-3 PUFA	0.51^{B}	0.48^{B}	0.56^{B}	1.50^{A}	0.20	<.0001
n-6/n-3	13.73^{A}	13.49^{A}	12.20^{A}	4.76^{B}	1.77	<.0001

The following FAs (found in percentage below 1% of total FAME) were utilised for calculating the Σ classes of FAs but they are not listed in the table: C13:0, isoC14:0, C14:1n-5, C15:0, anteisoC15:0, isoC15:0, C15:0, isoC16:0, C16:2n-4, C17:0, C17:1, C18:2n-4, C18:3n-6, C18:3n-3, C18:4n-3, C18:4n-1, C20:0, C20:1n-11, C20:1n-9, C20:4n-6, C20:5n-3, C22:1n-9, C22:1n-7, C22:6n-3.

A, B, C: $p < 0.01$; a, b: $p < 0.05$.

Abbreviations: RMSE, root mean square error; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 9. Fatty acids of the polar lipid fraction (g/100 g total fatty acid methyl esters) of BSFL freeze-dried larvae.

Group	Control	Beef	Chicken	Salmon	RMSE	*P*-value
C12:0	28.87	30.87	31.52	28.54	6.66	0.8838
C14:0	4.89	4.69	4.71	4.41	0.98	0.9199
C16:0	12.05	13.77	13.21	13.61	1.01	0.1035
C16:1n-7	2.51^{C}	4.44^{A}	3.81^{B}	3.70^{B}	0.22	<.0001
C18:0	4.50	3.37	3.28	3.92	0.68	0.0637
C18:1n-9	20.31	24.22	23.85	25.93	3.37	0.1647
C18:1n-7	0.74^{A}	1.09^{B}	0.69^{C}	1.11^{B}	0.17	0.0023
C18:2n-6	14.92^{A}	8.50^{B}	9.87^{B}	9.91^{B}	2.07	0.0025
C20:0	1.36	1.11	1.20	1.28	0.34	0.7137
C22:0	1.37	1.25	1.32	1.38	0.37	0.952
C22:5n-6	3.60^{a}	1.56^{b}	1.64^{b}	0.29^{b}	1.19	0.0117
SFA	54.99	56.36	56.39	54.47	6.04	0.9497
MUFA	24.32^{b}	30.83^{a}	29.46^{a}	32.02^{a}	3.53	0.0363
n-6 PUFA	18.71^{A}	11.55^{B}	12.97^{B}	11.47^{B}	2.63	0.0041
n-3 PUFA	1.16^{B}	0.82^{C}	0.82^{C}	1.61^{A}	0.17	<.0001
n-6/n-3	16.14^{A}	14.17^{A}	15.69^{A}	7.12^{B}	1.47	<0.0001

The following FAs (found in percentage below 1% of total FAME) were utilised for calculating the Σ classes of FAs but they are not listed in the table: C10:0, isoC14:0, C14:1n-5, anteisoC15:0, isoC15:0, C15:0, isoC16:0, C16:1n-9, C16:2n-4, C17:0, C17:1, C18:3n-6, C18:3n-3, C18:3n-4, C18:4n-3, C18:4n-1, C20:1n-11, C20:1n-9, C20:3n-6, C20:4n-6, C20:3n-3, C20:4n-3, C20:5n-3, C22:1n-9, C22:1n-6, C22:5n-3, C24:0, C22:6n-3.

A, B, C: $p < 0.01$; a, b: $p < 0.05$.

Abbreviations: RMSE, root mean square error; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

acid profile of the neutral lipid fraction (Table 8) indicated a higher ($p < 0.01$) lauric acid content in the larvae obtained on the CTRL substrate than the other groups and CD group had a higher value of that fatty acid than both the BD and the SD groups. A similar trend was observed for the total SFA. SD larvae had the highest ($p < 0.01$) n-3 PUFA content and the lowest n-6/n-3 ratio in comparison to the other groups. Regarding the fatty acid profile of the polar lipid fraction (Table 9), no differences were observed among the groups for the content of lauric acid and total SFA. CTRL group had the lowest ($p < 0.01$) value of MUFA and the highest ($p < 0.01$) of n-6 PUFA in comparison to the other groups. The n-3 PUFA showed the highest ($p < 0.01$) value in the SD group, followed by CTRL and then, together, by BD and CD groups. The SD group had the lowest n-6/n-3 ratio.

Discussion

The waste accumulation and its consequent management is a global concern and, among the diverse strategies to alleviate the problem, there is the possibility to reuse waste for strategic production (UN Environment Programme, 2024). In a context of circular economy, organic waste can gain a new life if used as substrate for insect larvae growing. Regarding the expired pet food, even if no data is available in literature, the producer indicated an annual average value of 0.1% of discarded products on the total production. When expired canned cat food was reused as a substrate for BSFL, all diets did not show the same effectiveness. Among the three tested diets, only the salmon-based formulation (SD) allowed the larvae to achieve growth performance comparable to those obtained with the control broiler diet (CTRL), which contained only vegetal ingredients.

In fact, compared to the other two cat foods (based on Beef and Chicken Diets, respectively), BSFL reared on SD had a shorter larval development time (20 *vs.* 22 days), and a higher final weight (+14.98 and +8.24% compared to BD and CD larvae, respectively). In addition, compared to the CTRL one, the larvae of the SD group had a lower (-19.7%) mortality rate. These results indicate that canned cat food can be successfully used as a substrate for BSFL, leading to satisfactory growth and development. However, despite the relatively similar chemical-nutritional composition of the tested pet food diets (BD, CD, SD), differences in larval performance were observed. This suggests that factors beyond basic nutrient composition, such as ingredient digestibility, presence of additives, or micronutrient bioavailability may influence larval growth and efficiency, in agreement with Abduh et al. (2022). A deeper analysis of these aspects is needed to better understand the observed variations and optimise substrate selection for insect rearing. In this study, the main difference among the three tested pet food diets was the type of animal protein source included (CD, BD or SD), each added in similar percentages within the formulation. Thus, in our opinion, the differences among the larvae growing on the diverse cat foods can be ascribed to a different possibility of

the larvae to utilise the BD, CD or SD protein sources. Bonelli et al. (2020) observed that midgut of BSFL is able to adapt to diets with different nutrient content and the result of this adaptation is the ability of these insects to grow on a variety of feeding substrates. However, the changes in the hindgut described by Bonelli et al. (2020) have primarily been studied under conditions of poor nutritional quality, and to date, no studies have examined whether similar adaptive mechanisms occur in the hindgut when larvae are reared on nutritionally adequate diets. The decrease in larval protein content observed with the BD substrate could be attributed in part to the lower protein content of the BD substrate itself. However, this result is consistent with findings from Addeo et al. (2021), who reported a similar protein reduction (14.88%–16.15% DM) in BSF larvae fed substrates containing increasing levels of butchery waste. Despite comparable protein contents in the substrates, larvae in that study accumulated significantly less protein than those reared on a vegetable or standard diet. This suggests that beyond crude protein concentration, other aspects such as the origin and protein digestibility, the presence and content of connective tissues, and processing conditions may have impaired nitrogen assimilation. Therefore, similar larval responses across both studies highlighted that protein quality and bioavailability, rather than quantity alone, are key determinants in larval nutritional outcomes. Larvae reared on chicken- and salmon-based substrates (CD and SD) exhibited higher protein content than those reared on the beef-based substrate (BD), confirming that the quality of the protein source in the substrate influences protein accumulation in the larvae (Barragán-Fonseca et al. 2018). The literature reports that the protein content of BSFL is highly dependent on the availability of essential amino acids in the growth substrate (Pimentel et al. 2017; Barragán-Fonseca et al. 2018). Overall, the results suggest that BSFL are able to use all the tested substrates giving interesting performance, but not all the growing substrates can be used in the same way. The analysis of larval performance revealed significant differences among experimental groups. In particular, larvae reared on salmon based diet exhibited the highest growth rate (0.010 g/day) and a lower mortality rate compared to other groups. As stated by Bonelli et al. (2020), the digestive plasticity of BSF allows adaptation to different diets, but nutrient digestion and assimilation vary depending on substrate quality. The enhanced growth performance of BSFL reared on fish-derived substrates has also been demonstrated in previous studies, including those by Li et al. (2022) and Camperio et al. (2025). In terms of protein quality, it is plausible that the fish offal provided a more favourable amino acid composition and greater digestibility. This could be due to the lower collagen content in fish compared to the waste streams from the chicken and beef supply chains, which typically contain more fibrous connective tissues, thus resulting less digestible. Furthermore, the salmon substrate was rich in long-chain PUFAs which may serve as significant energy sources for larvae, thereby promoting their growth (Li et al. 2022). Additionally, the Substrate Reduction Index (SRI) was higher in the SD and CD groups, suggesting greater efficiency in converting the substrate into larval biomass. This parameter is crucial for assessing the sustainability of insect farming in circular economic contexts, as reported by Meyer et al. (2021). During the larval stage in insects there is an accumulation of storage lipids in the droplet cells as a reserve for further use during other phases of insect life such as metamorphosis, flight, reproduction (Toprak 2020). Adult flies with higher lipid reserves are able to give better performance in terms of lifespan and reproductive activity in comparison to adult insects with a lower amount of storage lipids (Downer and Matthew 1976; Ziegler and Van Antwerpen 2006; Arrese and Soulages 2010). Despite the lipid content in CTRL substrate was more or less half than that of the other substrates, the lipid content of the larvae grown on the diverse substrates was not different except for larvae fed on BD that had a lower total lipid content. That is because, as for other insect species, the lipid synthesis in BSF is from carbohydrates (Gold et al. 2020; Franco et al. 2021; Carpentier et al. 2024), that represented a major component of dietary substrates also in our trial. However, despite the similar carbohydrate content of the other substrates, larvae on BD cat-food showed a lower amount of lipids stored in the body. This could be attributed to the low growth rate of BSF larvae and their reduced ability to convert the substrate, as previously discussed. In addition, both Tschirner and Simon (2015) and Eggink et al. (2022) highlighted the importance of the protein-to-lipid (PTL) ratio in the substrate, which influences larval growth, metabolism, and body composition. Specifically, the lower the PTL ratio, the lower the nutrient assimilation and overall larval development (Eggink et al. 2022). In line with this, the BD larvae, which were reared on the substrate with the lowest PTL ratio, also exhibited the lowest lipid content. If this low level of lipid reserves is not good in the perspective of a future adult insect, in terms of feed industry it could be an interesting

result because the high level of lipid in insect larvae can be a problem for the use in the formulation of animal diets (Motte et al. 2019) and very often a total or partial defatting of the insect meal is required before its use, significantly increasing the costs of production and the final price of the insect meal. Cholesterol was detected only in larvae grown on cat food diets. This result is not surprising because *H. illucens* larvae are not able to synthesise cholesterol (Tognocchi et al. 2024) due to the deficiency of enzymes for cellular synthesis, thus it is possible only an assimilation from the substrates during the feeding phase, in our case the larval stage. Insects require dietary cholesterol to satisfy their cellular, physiological, developmental, and reproductive needs (Haas et al. 2023). Despite it not being possible to measure the cholesterol content in the substrates, the present findings agree with other studies which confirmed the use of different growing substrates deeply modified the cholesterol content of BSFL as well as the overall sterol profiles (Boukid, 2021; Tognocchi et al. 2024). Lipid composition is critical in insects, particularly the presence of polyunsaturated lipids, as these are precursors for sex pheromones and play a crucial role in the cell membrane in maintaining homeviscosity at different temperatures (Boatta et al. 2023). In our trial, CTRL and the SD groups had a higher content of total PUFA than the other two groups. Protein-rich substrates, such as those derived from animal waste or fish offal, have been found to produce larvae with a FA profile richer in unsaturated fatty acids (Tschirner and Simon 2015). The balance between SFAs and UFAs seemed to be influenced by the protein-to-fat ratio of the substrate, and high protein content has been associated with an increase in the synthesis of long-chain unsaturated fatty acids, which are valuable for nutritional purposes (Purschke et al. 2017). Considering the potential use of BSFL in human nutrition, it is noteworthy that the SD substrate led to a significant increase in n-3 PUFA content, reflecting the influence of dietary fatty acid composition on the larvae's lipid profile. This confirms that the fatty acid composition of BSFL can be partially modulated by the substrate used for rearing. However, despite this shift, the overall SFA content remains high, which is an important aspect to consider when evaluating their nutritional value. Given the potential application of BSFL in both human and animal nutrition, particularly in aquaculture, various studies have investigated methods to enhance their n-3 PUFA content by modifying the composition of the rearing substrate. (Oonincx et al. 2020; Georgescu et al. 2022) or naturally rich sources, such as fish offal (Putra et al. 2021). However, the success of such enrichment strategies appears to depend on various factors, including the source of PUFA (e.g. substrate content, added oils), the larval developmental stage (Georgescu et al. 2022), and the genetic characteristics of the larval population (Tognocchi et al. 2024). In the present study, despite the differences in the n-3 PUFA levels of the rearing substrates, the larvae exhibited a similar relative abundance of this fraction, approximately 2 g/100 g of total FAME. Notably, the content of docosahexaenoic acid (DHA, C22:6n-3) in SD larvae did not directly mirror the salmon-based diet pet food. This observation aligns with findings by Li et al. (2022), suggesting that DHA (C22:6n-3) and eicosapentaenoic acid (EPA, C20:5n-3) may serve as significant energy sources for larvae, thereby promoting growth but reducing the accumulation of these valuable fatty acids. Conversely, enriching substrates with alpha-linolenic acid (C18:3n-3) has been shown to increase n-3 PUFA concentrations in larvae without negatively affecting their development (Georgescu et al. 2022). Finally, it is worth highlighting that this study identified the presence of C18:4 n-3 in the larvae at levels up to seven times higher than in the starting substrate. Notably, in the CTRL group larvae, this fatty acid accounted for more than 1% of the relative abundance, despite being absent in the reference substrate. This suggests that its presence is not solely dependent on dietary intake but rather on the intrinsic ability of BSFL to synthesise it *de novo* through elongation and desaturation pathways. Many insects, including BSFL, possess the enzymatic machinery necessary for lipid metabolism, allowing them to convert precursor molecules, such as shorter-chain SFA and MUFA, into a broader range of fatty acids through metabolic transformations (Barragán-Fonseca et al. 2018). In particular, insects can synthesise certain PUFAs and modify their fatty acid profiles through fatty acid elongases and desaturases, which catalyses the transformation of dietary lipids, or *de novo* synthesised fatty acids (Pimentel et al. 2017). This metabolic flexibility is crucial for BSFL that develop on substrates with varying lipid compositions, as it ensures sufficient energy storage and membrane functionality. Moreover, the observed synthesis of this FA in all substrate groups further supports the idea that BSFL maintain a relatively conserved lipid biosynthesis mechanism that is not strictly dictated by diet composition but rather regulated by internal metabolic needs. Such metabolic adaptability plays a key role in their ecological success and their potential as a sustainable protein and lipid

source in animal and human nutrition. Understanding these processes is essential when considering BSFL for feed applications, as their fatty acid composition can be tailored not only by dietary lipid content but also by leveraging their endogenous metabolic pathways. Similarly, Tognocchi et al. (2024) also reported the presence of EPA, which they described as unusual for this species. Lauric acid was always the most abundant fatty acid in the lipid of BSFL, irrespective of its content in the feeding substrates. However, it is possible to detect some important differences among groups with the highest content in the CTRL larvae and the lowest in BD larvae. BSFL are able to synthesise LA from the carbohydrates of the diet and several studies showed an increased level of LA with the increase of NFE content in the diet (Ewald et al. 2020; Hoc et al. 2020; Eggink et al. 2022). However, in the present trial the amount of NFE was very similar among the different substrates, thus it is logical to suppose that not only the amount but also the kind of carbohydrate can have a role in the *de novo* synthesis of lauric acid. In this regard, the CTRL diet was based on vegetable ingredients and part of its carbohydrates are structural carbohydrates, measured as crude fibre. In the tested cat food, carbohydrates are partly of vegetable origin (from rice and other vegetables) and partly of animal origin (glycogen residue); it was not possible in this trial to differentiate the different kinds of carbohydrates, but our results are strongly suggestive for a different use of carbohydrate sources by BSF to produce lauric acid. This point is very interesting and warrants further research, especially considering the potential industrial applications of insect larvae and their derivatives. Beyond their use in animal feed and human nutrition, insect-derived lipids are gaining interest in the cosmetics and personal care industry due to their bioactive properties and sustainability (Franco et al. 2021). Future studies should explore how different rearing conditions and substrates influence the quality and composition of insect lipids for such applications. Lauric acid shows a lot of interesting properties, since it is able to modulate blood cholesterol (Decker 1996) and exerts antimicrobial properties *in vivo* and *in vitro* (Dayrit 2015). SFAs, such as lauric acid and stearic acid, are the most represented fractions in triglycerides, as recently highlighted by Tognocchi et al. (2024). Although the fatty acid content differs between this study and the findings of Tognocchi et al. (2024), these variations can be attributed to factors such as substrate type and growth conditions. However, the relative abundance of individual fatty acids follows the same pattern described by Tognocchi et al. (2024). Specifically, SFA dominate the triglyceride fraction, followed by MUFA and n-6 PUFA. As observed by Tognocchi et al. (2024), this study also identified a low abundance of fatty acids with more than 20 carbon atoms. However, these long-chain fatty acids were found in the phospholipid fraction in both the present study and the paper by Tognocchi et al. (2024). A plausible explanation is the essential role of PUFA within phospholipids in maintaining cellular membrane fluidity. As reported by Tognocchi et al. (2024), this hypothesis was supported by the absence of substrate effects on the phospholipid fraction. In contrast, this study observed a significant influence of the substrate on the fatty acid profile of the phospholipid fraction, with the exception of SFAs that were found in similar quantities in larvae from different groups. All together the present findings suggest the need for further research to fully understand the lipid metabolism of this species, which would help optimise growth conditions and improve or modulate its nutritional profile.

Conclusions

BSFL can use a wide range of substrates for feeding and expired canned cat food can be a valuable growing substrate, giving growing performance competitive with the CTRL group, utilising a commercial broiler diet. However, diets containing high amounts of protein from animals of different origins can influence the chemical composition of BSFL. Nevertheless, the use of these substrates must be carefully evaluated, as current regulations do not authorise their use in insect rearing for feed or food purposes (EU Regulation No. 2017/893). Future research should consider these legal constraints when assessing the feasibility of different substrates for large-scale production. Among the tested substrates, BD resulted in the least favourable BSFL production, primarily due to its association with a reduced lipid accumulation in the larvae. Although the lauric acid content in BSFL remains high (accounting for 40–50% of total fatty acids), which is advantageous for certain industrial applications, it may be less desirable in some feed formulations. In contrast, the increase in n-3 PUFA content and the improved n-6/n-3 ratio observed in larvae reared on the salmon-based diet (SD) represent beneficial traits from a nutritional perspective.

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Ethical statement

Ethical approval is not required by national laws.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

Data will be made available on request.

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