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Valorization of Former Foodstuffs and Heat-Treated Post-Consumer Catering Waste through Black Soldier Fly Larvae

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Highlights:

- Pre-consumer former foodstuffs produced BSFL yields close to control.
- Post-consumer catering waste prolonged development and reduced survival.
- Packaging removal improved biological performance but requires aeration.
- Heavy metal contents were below EU feed limits; Cd showed the clearest transfer.
- Pilot scale (n=2 trays/diet) reports indicative ranges, not inference.

Abstract:

Former foodstuffs are promising substrates for black soldier fly larvae (BSFL; *Hermetia illucens*), but evidence from heterogeneous, industrially collected waste streams remains limited. This pilot experiment evaluated larval growth, bioconversion performance, composition, and heavy-metal transfer using four diets from an industrial waste handler: one post-consumer catering waste stream and three pre-consumer expired foodstuff streams differing in meat content and packaging removal. A moisture-matched broiler starter diet served as the control. The control produced the fastest growth and best feed conversion ratio (FCR 3.94; bioconversion rate 25.4%; harvest at 14 days). Pre-consumer streams most closely matched the control, yielding 1.84–2.08 kg per tray, FCRs 4.81–5.42, waste reduction 47.8–52.8%, survival 53.7–60.0%, and harvest at 16 days. Post-consumer catering waste performed poorest (survival 42.4%; waste reduction 26.8%; FCR 6.39; harvest at 33 days). Comparing identical substrate streams with and without packaging removal showed that compliance-driven packaging removal improved waste reduction (52.8% vs. 48.9%) and survival (60.0% vs. 56.8%) but slightly reduced yield, highlighting trade-offs between regulatory compliance and substrate aeration. BSFL biomass composition shifted with diet, notably fat and ash, while protein remained relatively stable. Cadmium, lead, zinc, and manganese concentrations in substrates and BSFL biomass were below EU feed limits, although cadmium showed the clearest transfer to biomass. Results indicate that pre-consumer former foodstuffs can be viable BSFL substrates under practical conditions, provided sorting, regulatory compliance, and batch variability are managed. Given the pilot scale (two trays per diet), outcomes are presented as indicative performance ranges rather than definitive estimates.

1 INTRODUCTION

The demand for sustainable protein production is rapidly increasing and is already exceeding current capacities (McKenzie & Williams, 2015). With continued global population growth projected over the coming decades (United Nations Department For Economic And Social Affairs, 2025), alternative protein sources and more efficient production systems are urgently needed. At the same time, conventional protein production exerts substantial environmental pressure, including deforestation for feed crops and overfishing. These processes contribute to biodiversity loss and increased greenhouse gas emissions (Food and Agriculture Organization of the United Nations, 2009). In parallel, global food waste has reached critical levels, with approximately 1.05 billion metric tons wasted in 2022, representing 19% of food available to consumers, and an additional 13% lost along the supply chain (United Nations Environment Programme, 2024). In this context, insect-based bioconversion has emerged as a promising strategy to valorise organic waste streams while also producing protein within a circular economy framework (Siddiqui et al., 2024).

Among candidate species, Black Soldier Fly larvae (BSFL), *Hermetia illucens*, are particularly suitable due to their high feed conversion efficiency, rapid development, and ability to process diverse organic substrates (Lalander et al., 2019; Siddiqui et al., 2022). The process yields protein- and fat-rich larval biomass as well as frass suitable for use as fertiliser (Eke et al., 2025; Lopes et al., 2022). BSFL have several advantages compared to other insect species used in insect bioconversion. The larvae can mature in as little as 21 days and tolerate substrates with high moisture content (up to 80–90%) with minimal performance loss (Lalander et al., 2020; Oonincx et al., 2015).

Numerous studies have shown that BSFL will process a wide variety of substrates, with their diet strongly influencing their bioconversion efficiency, nutritional value, development time and morphological characteristics (Eke et al., 2025; Ganesan et al., 2024; Lopes et al., 2022; Siddiqui et al., 2024). However, in the context of the European Union, only a limited proportion of food-derived waste streams are currently permitted for insect bioconversion since BSF is classified as a farmed animal approved as feed for farmed animals in the EU (Lambert & Cornish, 2023). To maintain feed safety, the EU has prohibited the use of mixed waste streams, former food containing meat or fish and household food waste, while plant-based former foodstuffs or side streams, dairy, honey, and egg products are allowed to be used in insect bioconversion (EFSA Scientific Committee, 2015; Lähteenmäki-Uutela et al., 2021).

Terminology used in this paper follows EU feed and waste distinctions. “Former foodstuffs” refers to food products originally intended for human consumption that are no longer marketable for commercial reasons and are collected from pre-consumer sources (e.g., retailers, manufacturers). “Post-consumer catering waste” refers to food waste generated after consumer handling (e.g., catering/restaurant waste) and is included in this study only as a technical performance reference. Where the term “waste stream” is used, it denotes the

logistical origin and heterogeneity of the collected material and does not imply legal eligibility as insect feed under EU rules.

Former foodstuffs originating from pre-consumer waste streams represent a promising substrate for insect bioconversion due to their high availability and low opportunity costs (Gligorescu et al., 2022; Pinotti et al., 2023). These former foodstuff streams are often heterogeneous in their nutritional composition and may contain residual packaging materials and variable levels of contaminants such as heavy metals, yet their impact on BSFL performance and safety remains insufficiently characterised. Moreover, whether mandatory packaging removal affects bioconversion performance in real industrial streams remains an open question.

The aim of this study was to evaluate the bioconversion potential of locally sourced former food substrates under conditions representative of real supply chains. Specifically, the study addressed the following research questions:

1. How do pre-consumer and post-consumer former food streams differ in bioconversion efficiency and larval performance when processed by BSFL?
2. Does the presence of residual packaging materials affect larval growth, survival, or biomass composition?
3. Are heavy metal concentrations in the resulting larval biomass within the regulatory limits established for animal feed in the European Union?
4. Does mandatory packaging removal affect bioconversion performance, and what are the implications for compliant industrial substrate management?

2 MATERIALS AND METHODS

The BSFL were reared at the Biotechnical Faculty, University of Ljubljana insect rearing laboratory. The crates were kept at 27 ± 2 °C and 70 ± 10 % relative humidity (RH) in the dark as described by Deruytter et al., 2023. The experiment was designed as a pilot study aimed at evaluating the feasibility and performance range of real-life former foodstuff streams under practical rearing conditions. Due to logistical and material constraints typical for industrial waste-derived substrates, each diet was replicated in two trays only. Consequently, the study does not provide true biological replication suitable for inferential statistical testing, and results should be interpreted descriptively.

2.1 Supply of substrates for rearing BSFL

Four different diets from former foodstuffs were used for rearing BSFL. The diets were not controlled for energy and nutritional content beforehand, since we wanted to test the effect of real substrates from an organic waste handling plant. The substrates used in the experiments were chosen to be ready to use from the current waste processing setup with as little interference as possible. Substrate collection was done on 09 Jul 2025.

Regulatory context of the tested materials: diets derived from pre-consumer former foodstuffs were collected as real industrial batches and were assessed as received to reflect real supply-chain heterogeneity, rather than being pre-screened or optimised prior to the pilot run. Diets containing visible packaging remnants (diets 2 and 3 and partially diet 4 before manual picking) represent non-compliant reference scenarios under EU feed rules due to physical contamination. Diet 1 (post-consumer catering waste) is not permitted as insect feed under current EU legislation and is therefore treated strictly as a technical reference for process feasibility and biological performance. The substrate from post-consumer catering waste was sampled after grinding and sterilisation. Thermal pretreatment of the food waste mix took place in an 8 m³ industrial autoclave (Monts, Hydrolyser H, Czech Republic). The food waste was autoclaved under pressure, at 3 bars for 20 min at the temperature 133 °C.

We collected pre-consumer expired foodstuffs from various grocery stores and distributors. The material was separated into two piles of about 1 metric ton each. One pile contained mixed former foodstuffs, and the other contained removed meat and meat products. Before processing the experimental substrates, the production line was flushed with approximately half of the available material to clear residues from previous batches. The remaining material was then processed and sampled into 50 L barrels. The barrel without meat and meat products was divided into 2 subsamples, from one subsample, the packaging material was separated by hand. All barrels were transferred from the waste processing site to the insect rearing laboratory immediately after the preparation of feeding substrates.

2.2 Preparation of the diets

We thoroughly mixed the feeding substrates and placed 5000 ± 10 g into each rearing crate. An additional 5000 ± 10 g per crate for refeeding was frozen at -20 ± 1 °C. The control group diet was prepared based on the average moisture content of the test substrates, measured using a digital moisture meter (Lutron MS 7004SD), by mixing dry mash meal for broilers (BRO-STARTER Jata Emona) with tap water pre-tempered to rearing chamber conditions. For refeeding, the frozen feeding substrates were thawed for 24 h in the insect bioconversion growth chamber to equilibrate the temperature. The control group feed was also prepared in the same way as the first time and tempered for 24 h. From each group, 500 g of feed mixture was sampled for nutritional, chemical and physical evaluation. The samples were stored at -20 ± 1 °C immediately after taking them, until the preparation for analyses.

2.3 Neonate nursing and inoculum preparation

Stabilized 0-DOL neonates were obtained from a commercial manufacturer, Nasekomo. We placed them on a fine mesh sieve above an empty crate inside mosquito mesh to minimise access by non-target species. The sieve remained above the crate for 30 minutes to recover viable neonates of similar activity from the stabilizing matrix to standardise collected cohort. 3 g of neonates were then transferred to 900 ± 10 g of control group feed mix and kept inside the mosquito mesh for 5 days at 27 ± 2 °C and 70 ± 10 % RH in the dark. After five days, the mixture was gently homogenised by decanting it between two crates 20 times. The quantity of

5-DOL was determined by counting the number of larvae in three randomly taken samples of at least 100 larvae. Ten subsamples of the 5-DOL and frass mixture, each containing ~15,000 larvae, served as inoculum for the trays with feed mixtures.

2.4 Experimental setup

The subsamples of larvae were inoculated on top of tempered feeding mixtures in pre-weighed stainless steel rearing trays (530 × 325 × 100 mm), which were randomly placed onto racks inside the rearing chamber. Between each stacked tray there was one empty rack to ensure proper ventilation. The top and bottom racks were filled with trays containing 10L of tap water to reduce boundary effects.

Timeline and naming of sampling days: neonates (0-DOL) were nursed for 5 days on the control feed to obtain a standardised 5-DOL cohort. Day 0 in this paper refers to the day of tray inoculation with 5-DOL larvae (i.e., larvae were 5 days old at inoculation). Subsequent intermediate measurements were performed on days 5 and 9 post-inoculation, followed by a diet-specific harvest when more than 10% of individuals had reached the prepupal stage.

2.5 Sample collection

Larval growth was monitored in two trays per diet. Tray-level measurements were used to summarize outcomes descriptively ($n = 2$). The average weight of larvae was determined on day 9 and at harvest. Samples were collected as described by Deruytter et al.(2023), to get the representative samples. A sample of 100 larvae was collected, washed, gently blotted dry with paper towels and weighed to determine wet mass. After weighing, larvae were inactivated by freezing at -20 °C and were not returned to the crates to prevent interference by potentially disturbing their performance.

Harvest time was determined separately for each diet based on daily visual checks and began when more than 10% of BSFL had transitioned to prepupae. At harvest, we recorded the tray weight. We separated the harvested material using a 10 mm sieve to remove packaging and large residues, then a 4 mm sieve to separate prepupae and larvae from frass. All fractions were weighed, then the sample of 100 larvae and prepupae was prepared and weighed in the same way as on days 5 and 9 after inoculation. To ensure a sufficient amount of material for analysis, an additional 250 g of harvested BSFL were washed, dried and immediately stored at -20 ± 1 °C until sample preparation for subsequent analyses.

Survival estimation: N_{start} was derived from the inoculum subsample counts. At harvest, N_{end} was estimated by counting larvae in multiple weighed subsamples of the harvested larval fraction and extrapolating to the total harvested larval mass per tray. This approach provides an operational estimate of survival and reflects both counting and subsampling uncertainty.

2.6 Nutritional analysis

Since all organic waste streams and BSFL have the potential to be used as feed substrates, we used proximate (Weende) analysis to determine their nutritional composition, as this method is commonly used in animal feed testing. The frozen samples were ground to granulation < 1

mm and lyophilized to prevent degradation of thermolabile components. The analysis was performed in-house at the Department of Animal Science, Chair of Nutrition. Dry matter was determined by AOAC Official method 934.01, crude protein by AOAC Official method 954.01, total fat content by AOAC Official method 948.15, crude ash by AOAC Official method 942.05, crude fibre by AOAC Official method 978.10. Nitrogen-free extract and organic matter content were calculated.

2.7 Heavy metal content analysis

Heavy metal content analysis was performed by the Agrochemical Laboratory, Agricultural Institute of Slovenia using their internal ICP-MS method to determine the presence and concentration of heavy metals in food and feed.

2.8 Calculations

Larval growth performance and diet bioconversion parameters were computed according to the formulas below. Definitions used in the calculations: “Substrate (input)” ($m_{\text{substrate(input)}}$) was defined as the total wet mass of feed added per tray over the full run (initial feed + refeed; 5000 g + 5000 g per tray). “Biomass gain (Yield)” was defined as harvested larval wet mass minus the estimated larval wet mass at tray start (inoculum). Unless stated otherwise, masses refer to tray-level wet mass; dry-matter-based values are explicitly indicated as DM.

$$\text{Yield (biomass gain):} \quad \text{Yield (g)} = m_{\text{larvae(end)}} - m_{\text{larvae(start)}}$$

$$\text{Dry weight content:} \quad \text{DW (\%)} = \frac{m_{\text{dry}}}{m_{\text{wet}}} \times 100$$

$$\text{Feed Conversion Ratio:} \quad \text{FCR} = \frac{m_{\text{substrate(input)}}}{\text{Yield}}$$

$$\text{Bioconversion rate:} \quad \text{BCR (\%)} = \frac{\text{Yield}}{m_{\text{substrate(input)}}} \times 100$$

$$\text{Waste Reduction:} \quad \text{WR (\%)} = \frac{m_{\text{substrate(input)}} - m_{\text{residual(end)}}}{m_{\text{substrate(input)}}} \times 100$$

$$\text{Survival rate:} \quad \text{SR (\%)} = \frac{No_{\text{larvae(end)}}}{No_{\text{larvae(start)}}} \times 100$$

$$\text{Protein Yield:} \quad \text{Protein Yield (g)} = \text{Yield} \times CP_{\text{larvae(WW)}}$$

$$\text{Protein efficiency ratio:} \quad \text{PER} = \frac{\text{Protein Yield}}{CP_{\text{substrate(WW)}} \times m_{\text{substrate(input)}}}$$

$$\text{Specific Growth Rate:} \quad \text{SGR (\%)} = \frac{\ln(m_{\text{larvae(end)}}) - \ln(m_{\text{larvae(start)}})}{t(\text{days})} \times 100$$

2.9 Data reporting and statistical considerations

No inferential statistical testing was performed due to limited replication (n = 2 trays per diet). Tray-level outcomes are presented descriptively. Where variability is shown (e.g., “±”), it represents the dispersion between the two tray observations and should be interpreted as an indication of the observed range rather than as a robust estimate of population variability.

3 RESULTS

Harvested biomass consisted predominantly of late-instar larvae, with a small proportion of prepupae at the time of harvest. For clarity and consistency, the term “BSFL biomass” is used throughout the Results and Discussion to refer to this harvested material, unless a specific developmental stage is explicitly discussed.

3.1 Larval growth performance

The experiment was concluded after 14 days for the control group, after 16 days for diets 2–4, and after 33 days for diet 1. The temporal growth trajectories of larvae reared on the different diets are shown in Fig. 1. Intermediate measurements of larval mass were performed on days 5 and 9 after inoculation. After the second weighing, the other part of substrate was added. Table 1 shows the mean individual larval mass at each sampling time per diet, calculated from the total mass of 100 larvae. The initial mass of 100 larvae on day 0 was the same for every tray and diet group, at 0.565 mg, corresponding to 0.00565 mg (5.65 μ g) per individual larva.

Table 1: Mean larval mass (mg) across two trays per diet; variability indicates the spread between the two tray observations ($n = 2$). BSFL reared on different diets, 0 = control, chicken feed mix; 1 = post-consumer catering waste; 2 = pre-consumer expired foodstuff without meat, ground with packaging; 3 = pre-consumer expired foodstuff with meat, ground with packaging; 4 = pre-consumer expired foodstuff without meat, packaging picked out after grinding.

Time of sampling	Average mass BSFL, reared on different diet (mg)				
	0	1	2	3	4
Day 5	24.5 $\pm$ 1.8	5.8 $\pm$ 0.1	89.9 $\pm$ 13.1	68.5 $\pm$ 1.8	118.1 $\pm$ 17.3
Day 9	186.3 $\pm$ 14.1	35.6 $\pm$ 0.7	137.4 $\pm$ 3.4	140.6 $\pm$ 1.9	149.9 $\pm$ 5.9
Harvest day	307.5 $\pm$ 8.4	246.6 $\pm$ 12.5	221.8 $\pm$ 17.3	258.1 $\pm$ 3.0	205.2 $\pm$ 10.7

Note: $n = 2$ trays per diet; “ $\pm$ ” denotes the difference between the two tray values (descriptive), not a robust SD estimate.

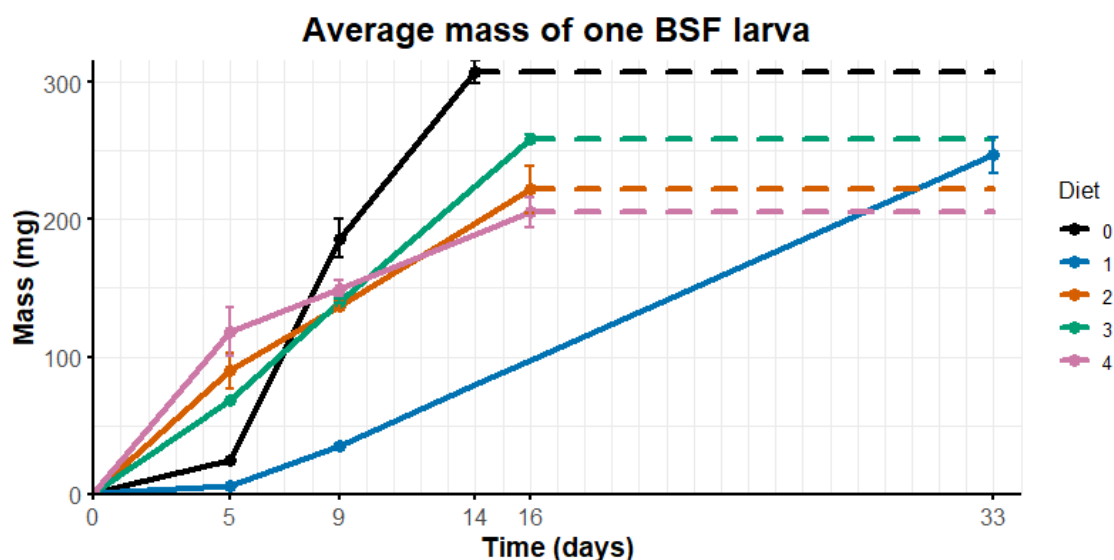


Fig. 1: Change in mean individual larval mass of BSFL reared on different diets, measured in 4 different time points. The last point for each diet represents the harvest day; a dashed line facilitates comparison of final larval mass across diets. Diets are

as follows: 0 = control, chicken feed mix; 1 = post-consumer catering waste; 2 = pre-consumer expired foodstuff without meat, ground with packaging; 3 = pre-consumer expired foodstuff with meat, ground with packaging; 4 = pre-consumer expired foodstuff without meat, packaging picked out after grinding. Values are descriptive (two trays per diet).

3.2 Nutritional values of different diets and BSFL reared on them

Nutritional values are expressed on a wet-mass basis, as substrates were tested under practical conditions without moisture correction, and on dry-matter basis for easier comparison between groups. Pooled samples of diets and BSFL were homogenised and analysed in duplicate; mean values are reported in Table 2 for diets and in Table 3 for BSFL. Differences in the nutritional composition of the experimental diets and the resulting BSFL biomass are illustrated in Fig. 2 and Fig. 3, respectively.

Table 2: Average wet-mass composition (g/kg) and average dry-mass composition (g/kg) of different diets, used as substrates for rearing BSFL, 0 = control, chicken feed mix; 1 = post-consumer catering waste; 2 = pre-consumer expired foodstuff without meat, ground with packaging; 3 = pre-consumer expired foodstuff with meat, ground with packaging; 4 = pre-consumer expired foodstuff without meat, packaging picked out after grinding.

Component (g/kg WW)	Diet				
	0	1	2	3	4
Dry matter	319.0	321.6	252.2	270.7	239.1
Water	681.0	678.4	747.8	729.3	760.9
Crude ash	14.5	18.4	36.1	47.4	22.1
Crude fat	24.9	35.6	20.1	23.8	15.3
Crude protein	19.9	98.7	37.4	37.9	25.0
Nitrogen free extract	81.0	70.0	36.2	36.1	31.0
Organic matter	178.8	99.0	122.4	125.6	145.7

Component in DM (g/kg DM)	Diet				
	0	1	2	3	4
Crude fibre	45.3	57.2	143.2	174.9	92.6
Crude ash	78.1	110.7	79.5	87.8	64.1
Crude fat	62.3	306.8	148.4	140.1	104.6
Crude protein	253.8	217.6	143.7	133.2	129.5
Nitrogen free extract	560.5	307.8	485.2	463.9	609.2
Organic matter	921.9	889.3	920.5	912.2	935.9

Note: Dry matter = crude ash + organic matter; organic matter = crude fat + crude protein + nitrogen-free extract. Values may not sum exactly due to rounding.

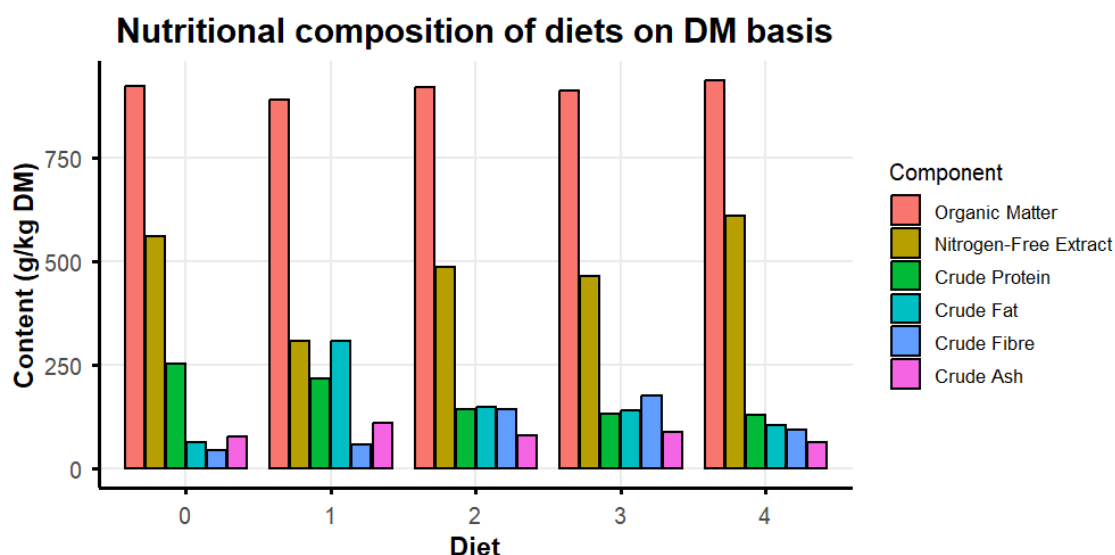


Fig. 2: Nutritional composition of different diet substrates, used for rearing BSFL; 0 = control, chicken feed mix; 1 = post-consumer catering waste; 2 = pre-consumer expired foodstuff without meat, ground with packaging; 3 = pre-consumer expired foodstuff with meat, ground with packaging; 4 = pre-consumer expired foodstuff without meat, packaging picked out after grinding.

Table 3: Average wet-mass composition (g/kg) and average dry-mass composition (g/kg) of BSFL, reared on different diets, 0 = control, chicken feed mix; 1 = post-consumer catering waste; 2 = pre-consumer expired foodstuff without meat, ground with packaging; 3 = pre-consumer expired foodstuff with meat, ground with packaging; 4 = pre-consumer expired foodstuff without meat, packaging picked out after grinding.

Component (g/kg WW)	BSFL, reared on diet				
	0	1	2	3	4
Dry matter	339.7	336.6	312.5	319.8	308.6
Water	660.3	663.5	687.5	680.2	691.4
Crude ash	32.7	46.3	29.2	28.2	26.0
Crude fat	110.2	125.5	132.0	127.7	124.1
Crude protein	144.4	124.0	105.7	113.6	107.0
Nitrogen free extract	52.4	40.8	45.6	50.4	51.5
Organic matter	307.0	290.3	283.3	291.7	282.6

Component in DM (g/kg DM)	BSFL, reared on diet				
	0	1	2	3	4
Crude ash	96.2	137.5	93.5	88.0	84.4
Crude fat	324.4	372.9	422.5	399.1	402.2
Crude protein	425.1	368.4	338.2	355.2	346.7
Nitrogen free extract	154.3	121.2	145.8	157.7	166.8
Organic matter	903.8	862.5	906.5	912.0	915.6

Note: Dry matter = crude ash + organic matter; organic matter = crude fat + crude protein + nitrogen-free extract. Values may not sum exactly due to rounding.

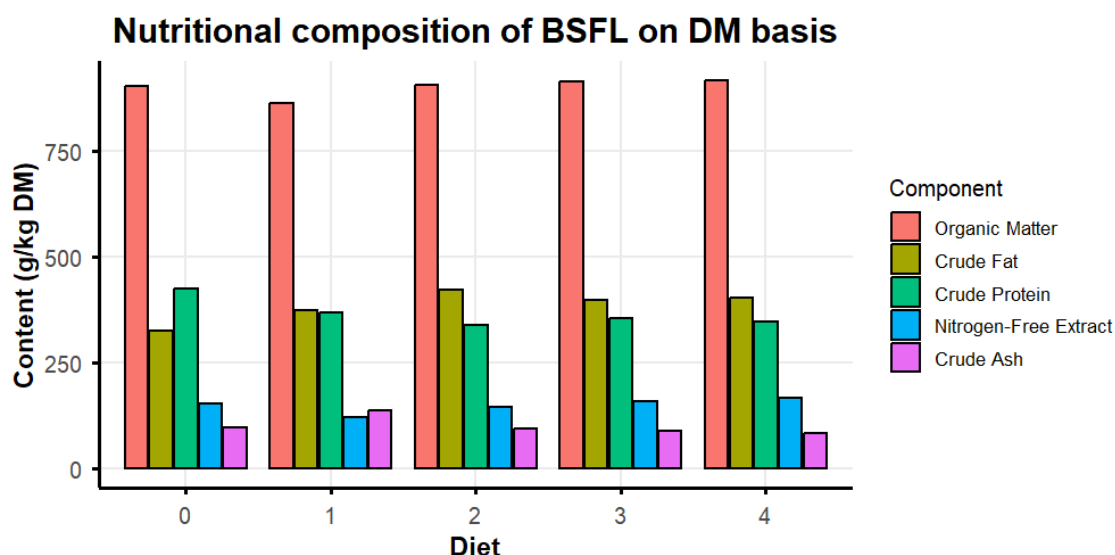


Fig. 3: Nutritional composition of harvested BSFL reared on different diets; 0 = control, chicken feed mix; 1 = post-consumer catering waste; 2 = pre-consumer expired foodstuff without meat, ground with packaging; 3 = pre-consumer expired foodstuff with meat, ground with packaging; 4 = pre-consumer expired foodstuff without meat, packaging picked out after grinding.

3.3 Bioconversion rate of diets by BSFL

The Table 4 shows the average values of bioconversion parameters from 2 trays per diet, n = 2 trays per diet; values are descriptive, and “±” reflects variation between the two trays.

Table 4: Bioconversion parameters of BSFL, reared on different diets, 0 = control, chicken feed mix; 1 = post-consumer catering waste; 2 = pre-consumer expired foodstuff without meat, ground with packaging; 3 = pre-consumer expired foodstuff with meat, ground with packaging; 4 = pre-consumer expired foodstuff without meat, packaging picked out after grinding.

Parameter	Diet				
	0	1	2	3	4
Yield	2537.17 ± 42.07g	1566.00 ± 62.58g	1887.46 ± 111.90g	2079.12 ± 14.91g	1844.46 ± 55.80g
Dry weight content	34.0%	33.7%	31.3%	32.0%	30.9%
Feed Conversion Ratio	3.94 ± 0.07	6.39 ± 0.26	5.31 ± 0.31	4.81 ± 0.03	5.42 ± 0.16
Bioconversion rate* (biomass gain)	25.4 ± 0.4%	15.7 ± 0.6%	18.9 ± 1.1%	20.8 ± 0.1%	18.4 ± 0.6%
Waste Reduction	47.1 ± 0.4%	26.8 ± 0.6%	48.9 ± 1.1%	47.8 ± 0.2%	52.8 ± 0.4%
Survival rate	55.0 ± 0.6%	42.4 ± 0.5%	56.8 ± 1.1%	53.7 ± 0.2%	60.0 ± 1.3%
Protein Yield	366.38 ± 6.08g	194.19 ± 7.76g	199.51 ± 11.83g	236.20 ± 1.69g	197.37 ± 5.97g
Protein efficiency ratio	0.45 ± 0.01	0.28 ± 0.01	0.55 ± 0.03	0.65 ± 0.00	0.64 ± 0.02
Specific Growth Rate	77.9 ± 0.2%	32.4 ± 0.2%	66.1 ± 0.5%	67.1 ± 0.1%	65.6 ± 0.3%

* Bioconversion rate is expressed as biomass gain relative to total substrate input (wet mass basis).

3.4 Heavy metals

Across all diets and BSFL samples, Cd and Pb concentrations were below the EU maximum levels for undesirable substances in feed (limits expressed at 12% moisture) as set by European Commission Regulation (EU) No 1275/2013 (2013). Zn and Mn concentrations were also below the EU maximum total contents permitted in complete feed (Zn: 120–180 mg/kg depending on species class; Mn: 100 mg/kg for fish and 150 mg/kg for other species). Heavy metal contents of diets and BSFL on dry-matter basis are shown in Table 5.

Table 5: Heavy metal content in the dry mass of tested diets, used for rearing BSFL and in the dry mass of BSFL. 0 = control, chicken feed mix; 1 = post-consumer catering waste; 2 = pre-consumer expired foodstuff without meat, ground with packaging; 3 = pre-consumer expired foodstuff with meat, ground with packaging; 4 = pre-consumer expired foodstuff without meat, packaging picked out after grinding.

Heavy metal content (mg/ kg DM)	Diet				
	0	1	2	3	4
Mn	76	16	53	34	34
Zn	97	36	25	23	24
Cd	0.07	0.09	0.04	0.04	0.05
Pb	0.11	0.23	0.75	0.49	0.44
Heavy metal content (mg/kg DM)	BSFL, reared on diet				
	0	1	2	3	4
Mn	82	10	45	23	41
Zn	35	14	23	18	23
Cd	0.08	0.14	0.06	0.03	0.07
Pb	0.07	0.04	0.36	0.21	0.31

4 DISCUSSION

4.1 Substrate composition

All tested substrates exhibited high moisture contents (68–76%). The control diet was adjusted to match the moisture level of the post-consumer catering waste, as both substrates showed comparable consistency. Such moisture levels are typical of food waste streams and remain sufficiently low to avoid the need for additional active ventilation, provided that standard rearing-chamber ventilation is applied, as reported previously (Lalander et al., 2020).

The control diet (0) showed the lowest crude fat content ($\approx 6\%$ of DM), whereas post-consumer catering waste (1) exhibited the highest crude fat content ($\approx 31\%$ of DM) and the highest ash fraction ($\approx 11\%$ of DM). Excess fat was visually apparent in the rearing trays, where separation of fat at the substrate surface was observed. Such fat accumulation likely reduced substrate aeration and contributed to the lower survival rates recorded for this diet. Similar observations were made in other studies (Sprangers et al., 2017), where prolonged development time and the need for structural substrate adjustments were reported under high-fat conditions.

The control diet (0) had the highest crude protein content ($\approx 25\%$ of DM). Among the test substrates, post-consumer catering waste (1) had the highest crude protein content ($\approx 22\%$ of

DM) and the lowest nitrogen-free extract content ($\approx 31\%$ of DM). This is consistent with previous studies on BSFL (Barragan-Fonseca et al., 2019; Cammack & Tomberlin, 2017) showing that bioconversion performance is not determined by protein or carbohydrate content alone, but rather by their ratio and the overall nutritional quality of the substrate. Diets with intermediate protein-to-carbohydrate ratios have been linked to higher larval yield, survival, and feed-use efficiency than diets shifted strongly toward either protein or carbohydrates.

The two ground, packaging-containing former foodstuff diets (2 and 3) had moderate crude fat ($\approx 14\text{--}15\%$ of DM) but the highest crude fibre ($\approx 14\text{--}18\%$ of DM). Packaging removal after grinding (diet 4) lowered crude fibre compared with the packaging-containing diets. The crude fibre fraction obtained using the Weende method is operationally defined as the residue remaining after sequential acid and alkali digestion. The procedure does not distinguish plant cell wall polymers from other acid- and alkali-resistant materials. As a result, synthetic polymers from packaging contamination may remain in the residue and artificially increase the measured fibre fraction (Schaller, 1978).

The post-consumer catering waste (1) had the highest ash fraction ($\approx 11\%$ of DM); all other diets had ash content of $6\text{--}9\%$ of DM. The high ash content could result from high salt content in the post-consumer catering waste, which could also influence growth, survival rate, and yield (Cho et al., 2020). In the case of pre-consumer waste, packaging contamination could also increase the ash value, since ash content is determined as residue after ignition. Non-flammable packaging (i.e. glass fragments and tins) could significantly influence the ash content, whereas plastic materials and paper or cardboard coatings are largely combustible and would therefore contribute less to the measured ash fraction (Netsch et al., 2025).

4.2 Regulatory framework

From a regulatory perspective, the use of former foodstuffs as insect-rearing substrates falls within the general EU feed safety framework, under which farmed insects are classified as food-producing animals. Consequently, both substrates and the resulting insect biomass must comply with the principles set out in Regulation (EC) No 178/2002 and Regulation (EC) No 767/2009. These regulations require that feed materials placed on the market are safe, unadulterated, and free from physical hazards.

The presence of packaging materials provided structure and increased aeration, which had beneficial effects on development rate, similar to the aeration benefits of cellulose fibre additions reported in other experiments (Yakti et al., 2023). Regardless of any potential technological or biological effects on larval performance, the presence of packaging residues in feed materials constitutes regulatory non-compliance under EU feed law. Regulation (EC) No 767/2009 explicitly prohibits packaging residues or parts thereof as physical contaminants in feed materials. This prohibition is qualitative and risk-based, without numerical tolerance thresholds. Consequently, former foodstuff substrates containing residual packaging cannot be considered compliant feed inputs under current EU legislation. Such materials should

therefore be regarded as transitional or experimental rather than legally marketable substrates for insect rearing.

It is important to distinguish this regulatory framework from legislation addressing biological and sanitary risks. Regulation (EC) No 1069/2009 and Commission Regulation (EU) 2017/893 primarily regulate biological and sanitary risks associated with animal by-products and processed animal proteins. These instruments do not address physical contaminants such as packaging residues. Physical contamination is addressed exclusively under the general feed safety provisions of Regulation (EC) No 767/2009.

The post-consumer catering waste included in this study served exclusively as a technical reference. Under current EU legislation, catering waste is explicitly excluded from the definition of former foodstuffs and is not permitted for use as feed for farmed animals, including insects (Commission Regulation (EU) No 142/2011, 2011; Regulation (EC) No 1069/2009, 2009). Therefore, results obtained for this substrate should be interpreted only in terms of biological performance and process feasibility, rather than as an assessment of regulatory suitability under EU conditions.

4.3 Larval growth performance

The fastest growth on the control diet was likely linked to its more balanced nutrient composition. BSF studies report highest performance at protein:carbohydrate ratios of about 1:2 to 1:3 (Eggink et al., 2023). Only the control diet was within this range; post-consumer catering waste had a P:C $\approx$ 0.7, and pre-consumer diets ranged from 0.21 to 0.29, indicating a greater carbohydrate bias. This may partly explain the slower growth observed in these groups. At the same time, residual packaging material in diets 2–4 may have improved substrate structure and aeration, which could have partly offset the less suitable nutrient balance (Nayak et al., 2023; Yakti et al., 2023). Despite a more moderate P:C ratio, diet 1 performed poorest overall, reflecting the overriding negative effects of excess fat and high salinity on substrate aeration and larval survival.

The notably higher individual larval mass observed in diet 4 at day 5 compared to diets 2 and 3 may reflect improved substrate accessibility following manual packaging removal, which could have facilitated earlier and more uniform feeding. The subsequent convergence across pre-consumer diets by day 9 suggests that this initial advantage diminished as larvae penetrated deeper into the substrate regardless of its physical structure. The slower early growth on the control diet, despite its superior nutritional profile, likely reflects an adaptation period before larvae fully colonised the more homogeneous substrate.

The best feed conversion ratio was observed in the control group (0), followed by BSFL reared on pre-consumer former foodstuffs (2–4), which also translated directly into biomass gain. The highest waste reduction was observed in BSFL reared on pre-consumer expired foodstuffs without meat with packaging sorted out after grinding (diet 4), and the lowest in post-consumer catering waste, likely due to higher salinity and sub-optimal consistency, which

prevented proper aeration of the substrate and led to lower survival rate. In this case, the substrate was also not fully processed by the end of the experiment. Packaging material contributed to aeration in pre-consumer former foodstuff, but larger pieces of packaging are less likely to be ingested and degraded under rearing conditions (Lievens et al., 2023).

The comparison of diet 2 and diet 4 represents the most controlled contrast in this study and, to our knowledge, the first empirical quantification of the performance consequences of compliance-driven packaging removal in a real industrial former foodstuff stream. Both diets were derived from the same source material without meat, differing only in whether packaging was removed after grinding; the only within-study comparison in which a single variable was isolated. Packaging removal improved waste reduction (52.8% vs. 48.9%) and survival (60.0% vs. 56.8%), suggesting that a cleaner substrate supported more complete processing and better larval health. However, FCR was marginally worse for diet 4 (5.42 vs. 5.31) and total yield was slightly lower (1844 g vs. 1887 g), which may reflect the loss of structural aeration previously provided by packaging remnants. Taken together, these results suggest that packaging removal has a net positive effect on biological performance and is mandatory under EU feed law, but that substrate aeration; currently provided incidentally by packaging; may need to be managed by other means in a compliant industrial setting.

4.4 Larval composition

Dietary composition was reflected in the composition of harvested BSFL biomass to some extent, especially in the case of crude ash and, consequently, organic matter content. The crude ash content was highest in BSFL biomass reared on post-consumer catering waste (1) and lowest in BSFL biomass reared on pre-consumer former foodstuffs (2–4), with especially low values in the diet from which packaging material was removed. Similar observations can be made in the case of crude fat content: except for post-consumer catering waste (1), the other diets' crude fat content was reflected in the BSFL biomass composition to some extent. These observations are aligned with findings from the literature (Spranghers et al., 2017).

The crude protein content and nitrogen-free extract were less dependent on the initial diet composition (Spranghers et al., 2017); the highest ratios were observed in BSFL biomass from the control diet (0) and from post-consumer catering waste (1). The lowest values were found in BSFL biomass from pre-consumer former foodstuff streams (2–4). If we consider the protein efficiency ratio as a bioconversion parameter, pre-consumer diets showed higher PER values than the control. This primarily reflects the lower crude protein content of these substrates rather than superior biological conversion efficiency per se, as the higher PER is partly a mathematical consequence of the smaller protein denominator. Protein yield in absolute terms remained highest in the control group, underlining that PER and protein yield convey complementary rather than equivalent information about bioconversion performance.

4.5 Heavy metals

With respect to the analysed heavy metals, all tested feeding substrates were below the current EU maximum levels for feed, which suggests a low chemical risk at the substrate level.

In our study, Cd was the only element that showed a consistent tendency to accumulate in BSFL biomass, whereas Pb, Zn, and Mn did not show accumulation under the tested conditions. This pattern is in line with earlier BSF studies (Diener et al., 2015; Fels-Klerx et al., 2016; Gligorescu et al., 2022), reporting Cd accumulation but low retention of Pb and Zn, and with more recent production-scale data confirming Cd bioaccumulation in BSF while not finding Pb bioaccumulation in practical, non-spiked substrates. It may be hypothesised that residual packaging materials could act as a secondary cadmium source by promoting leaching into the organic fraction during mechanical and thermal pretreatment, thereby increasing bioavailability, though this remains to be confirmed experimentally. In the present study, Pb concentrations in BSFL biomass were consistently lower than in the corresponding substrates across all diets, confirming the absence of Pb bioaccumulation under practical conditions. Notably, absolute Pb levels in larvae did not mirror substrate Pb levels proportionally across diets, suggesting that factors beyond substrate concentration; such as substrate matrix and larval feeding behaviour; may modulate Pb retention. At the same time, results from spiked-substrate experiments indicate that metal transfer may vary with substrate type and contamination level. Taken together, the present data suggest that Cd should remain the main element of concern in BSF safety assessments, as the measured BSFL biomass concentrations remained within current EU feed limits for the analysed metals (Commission Regulation (EU) No 1275/2013, 2013). From a regulatory standpoint, these results indicate that chemically compliant former foodstuffs may still require careful monitoring for physical contaminants, reinforcing the need for integrated safety assessments that combine both chemical and physical hazard evaluations.

4.6 Industrial implications

The performance contrast between diet 2 and diet 4 is the central practical finding of this study. Packaging removal is not optional under EU feed law; Regulation (EC) No 767/2009 explicitly prohibits packaging residues in feed materials; yet this study shows that removal eliminates an incidental source of substrate aeration that measurably supports larval survival and waste reduction. Compliant industrial systems must therefore compensate for this loss through deliberate aeration management. Potential approaches include addition of permitted structuring materials, controlled substrate mixing during rearing, or adjustment of moisture content and tray loading density. Quantifying the aeration requirements of packaging-free former foodstuff substrates should be a priority for future applied research in this area. Additionally, the observed variability between real-life substrate batches underscores the need for rapid quality screening (e.g., dry matter, fat content, and salinity proxies) to ensure stable process performance. From a safety perspective, cadmium should be prioritised in routine monitoring programmes, as it showed the clearest transfer from substrate to larval biomass under practical conditions.

4.7 Limitations

A key limitation is the pilot-scale design with two trays per diet, which restricts statistical inference and generalisation beyond the tested batches. The intent was therefore exploratory:

to document feasibility and the magnitude of performance differences under practical, heterogeneous former foodstuff streams, rather than to test formal hypotheses. Future work should include greater biological replication and repeated substrate collections across seasons and batches. In practice, only properly sorted, legally eligible pre-consumer former foodstuffs without physical contaminants qualify as feed-compliant substrates for insect rearing under current EU legislation.

5 CONCLUSIONS

This pilot case study, based on real former foodstuff streams collected under practical conditions, supports the use of black soldier fly bioconversion to convert low-opportunity-cost organic side streams into protein-rich biomass within a short rearing period. Given the limited replication ($n = 2$ trays per diet), findings should be interpreted as indicative ranges rather than definitive performance estimates. Although the control diet supported the fastest growth and best feed conversion, the pre-consumer former foodstuff diets produced results close to the control in terms of biomass production, waste reduction, and harvested BSFL biomass composition, while post-consumer catering waste performed less favourably. From a circular economy perspective, former foodstuffs are particularly suitable for BSF rearing because they have limited alternative value and can be upgraded into higher-value feed materials rather than being managed lower in the waste hierarchy. The heavy metals analysed remained below current EU feed limits in both substrates and harvested BSFL biomass. The direct comparison of identical substrate streams with and without packaging removal; the only single-variable contrast in this pilot; showed that compliance-driven packaging removal improved biological performance overall, while revealing that the resulting loss of substrate aeration must be actively compensated in industrial systems. However, cadmium should remain subject to monitoring, as some carry-over into insect biomass was observed. While this study reflects only one collection point and the summer period, the results indicate that properly sorted, legally eligible former foodstuff streams could be used more widely for industrial BSF production. From a regulatory perspective, these results align with the current EU framework governing insect-derived feed materials: biological composition is subject to specific regulatory limits, whereas the prevention of physical contamination relies on general feed safety principles rather than substance-specific thresholds. Overall, BSF rearing appears most justified when it upgrades former foodstuff streams with low alternative value into useful feed biomass, provided that substrate sorting, legal compliance, and batch variability are carefully managed.

CRedit authorship contribution statement

T. Ratajc: Conceptualization; Methodology; Investigation; Formal analysis; Visualization; Writing – original draft.

L. Bonin: Investigation; Writing – review & editing.

L. Lavrič: Resources; Data curation; Writing – review & editing.

E. L. Karara Geršak: Investigation, Writing – review & editing.

A. Kuhar: Conceptualization, Supervision; Project administration; Funding acquisition; Writing – review & editing.

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Declaration of competing interests

L. Lavrič is employed by KOTO Ltd., which supported this work. The remaining authors declare that the research was conducted in the absence of any other commercial or financial relationships that could be construed as a potential competing interest.

Data availability

The data are not publicly available due to commercial sensitivity but are available from the corresponding author upon reasonable request and with permission of KOTO Ltd., Ljubljana, Slovenia.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the authors used Microsoft Copilot and ChatGPT to improve language clarity and readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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