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Bridging lab and industry: The impact of a bio-conversion unit on black soldier fly larvae production and microbiome dynamics

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Abstract

Background: This study evaluates the viability of a novel bio-conversion unit (BCU) for extensive black soldier fly larvae (BSFL) production as a sustainable feed additive for livestock. The BCU's effectiveness in converting organic byproducts into valuable biomass via the production of BSFL was assessed to reduce reliance on environmentally taxing feed sources like soy and fishmeal. Additionally, the BCU was tested for its ability to replicate small-scale BSFL experiments in a realistic industrial environment while facilitating simultaneous testing of multiple feeding substrates for BSFL. BSFLs were reared in the BCU on various low-bioburden commercial byproducts, and their yield, macronutrient and micronutrient profiles were compared to those of larvae raised on a nutritionally balanced diet. High-throughput amplicon sequencing was used to investigate the impact of different diets on the BSFL gut microbiome, replicating laboratory findings on an industrial scale.

Results: Larvae reared on low-bioburden substrate in the BCU demonstrated comparable or improved protein and fat content compared to those reared on animal feed, with consistently high yields across all byproduct substrates. Micronutrient analysis revealed elevated calcium levels (compared to the literature), among other essential elements, in the byproduct-fed larvae, further supporting their potential as a nutritious livestock feed additive. Microbiome analysis confirmed a stable core microbiome across all conditions, with some genera emerging as dominant at the industrial scale, highlighting the importance of larger-scale replication for accurate microbiome research.

Conclusion: In this pilot study, the BCU proves to be a reliable and efficient system for broad BSFL production, converting organic byproducts into high-quality biomass suitable for animal feed. Its controlled environment and scalability make it a valuable tool for conducting industrial-scale scientific research on BSFL, bridging the gap between laboratory studies and real-world applications.

KEYWORDS
bio-conversion unit (BCU), black soldier fly larvae (BSFL), BSFL microbiome, insect protein production, sustainable feedstock, waste valorisation

INTRODUCTION

The black soldier fly (*Hermetia illucens*) (BSF) is a member of the soldier fly family (*Stratiomyidae*) and of the order of true flies (Diptera).[1] It is the most widespread member of the Stratiomyidae family, with a range spanning temperate, tropical and subtropical regions.[2] As far back as 1919, Lindner proposed that the larvae of insects such as the BSF could be fed on organic byproducts to recover fats and nutrients from waste food.[3] This is partly due to the high-fat content of the larvae of insects, with the BSF larvae (BSFL) being recorded to be 42%–45% crude protein and 31%–35% crude fat by dry weight.[4,5] This makes the BSFL a good source of both fat and protein and, therefore, a possible feed additive for animals or even as food for humans. These beneficial nutrient contents are further compounded by the fact that BSF is not known as a disease carrier,[6] unlike the common housefly (*Musca domestica* L, another candidate as an insect food source). Furthermore, in their adult stage, BSF requires minimal nutrients to survive, relying primarily on the nutrients they accumulated during their larval stage and needing only water.[6] Given the high nutrient content of BSFL and their non-disease-carrying nature, they present a promising alternative to traditional animal feed sources, especially considering the growing global demand for animal products and the environmental concerns associated with conventional feed options like soy.

With an ever-increasing demand for animal products worldwide, there is a growing need for alternatives to animal feed. Poultry is a popular and prominent choice source for animal products, with the total number of birds reaching >33 billion in 2020.[7] Feeding these birds is often done using soy meal or soy-derived products. This is because soy is high in protein and essential nutrients chickens need, being ∼42% protein and ∼47% lipids by weight.[8] However, the majority of soybeans are not only imported but also genetically modified (GM), with the EU importing ∼14 million tons of soybeans each year, 50% of which is from the United States, with 94% of the soybeans planted in the United States being GM.[9] Soybean farming in South America has also led to considerable deforestation, land appropriation and disputes, affecting climate change and local people's lives.[10] The BSFL is one of many suggested alternative livestock feed additives to combat the environmental cost of importing soybeans. BSFL have already been studied as a feed additive for fish (larvae fed manure and supplemented with fish offal),[11] broiler quail (defatted BSFL as a supplement to soy)[12] and most relevant to this study, broiler chickens (as a supplement of 250 g per Kg defatted BSFL to chicken feed (CF)).[13] In all observed studies, BSFL proved a suitable supplement/addition to livestock feed as a source of protein and essential amino acids. In chickens, BSFL meals have also been shown to replace fishmeal with no adverse effects on carcass weight, with the added benefit of (potentially) reducing the cost of chicken feeding by 23% per chicken.[14]

The nutritional suitability of BSFLs as a feed additive, combined with their comparatively low cost, would alone be the reason for adoption. However, the larvae can also be reared on organic byproducts, removing the cost of expensive substrate rearing. Currently, in the UK, BSFLs fed on 'waste' (manure, used cooking oil and all animal byproducts) are not allowed into the food chain with livestock. However, they can be fed on 'surplus' fruit/vegetables, brewers' grains and a limited selection of animal products such as dairy, eggs and blood (provided the produced BSFL are not fed back to the animal of origin).[15]

There has been a sizeable body of work on the factors affecting the growth and performance (weight gain, nutrient content, etc.) of BSFL under various conditions and on multiple substrates,[16,17] including experiments studying the microbiome of the BSFL and the effect of substrate on its composition.[18] However, most of these were small, laboratory-scale experiments, and the authors often voiced concerns that these results and BSFL performance may decrease or change at larger scales. Despite this significant body of work, most previous reports lack scale and consistency. There is no agreed-upon method of rearing (for example, rearing in a greenhouse[19] vs. rearing BSFL in the lab) and no consistent temperature, humidity and timescale for studies. As such, this paper presents a device and potential methodology for studying BSFL, its nutrients and microbiome at an industrial scale while maintaining consistent environmental conditions.

This study aimed to validate using a novel bio-conversion unit (BCU) system as a platform to study the BSFL microbiome and replicate work on the BSFL microbiome that was previously only performed at a laboratory scale while monitoring macro and micronutrient content. Simultaneously, the study aimed to present an efficient waste valorisation system using the BCU in tandem with widely available, low-bioburden organic byproducts. Furthermore, we hoped to demonstrate that the BSFL produced (at a larger scale) within the BCU were nutritionally viable as an animal feed additive.

METHODS

Design and development of the bio conversion unit (BCU)

In 2017, Inspro Ltd. was founded to sustainably valorise organic byproducts using black soldier fly larvae (BSFL). Recognising the environmental and economic challenges associated with transporting bulky organic matter over long distances, a dispersed, modular and scalable approach was adopted for the bioconversion process. This design allowed for local adaptation based on the availability of substrates and demand for the products, such as feed and fertiliser.

The BCU utilised free-standing plastic trays to reduce cost and increase flexibility. These plastic trays were designed to stack to promote horizontal ventilation across the substrate surface, preventing crust formation while facilitating the removal of gases such as methane and ammonia and ensuring adequate oxygen delivery to the larvae.

The Heating, Ventilation, and Air Conditioning (HVAC) system (Figure 1) was a critical component of the BCU, enabling the units to operate at full capacity while maintaining controlled environmental conditions. The HVAC system regulated oxygen levels, relative

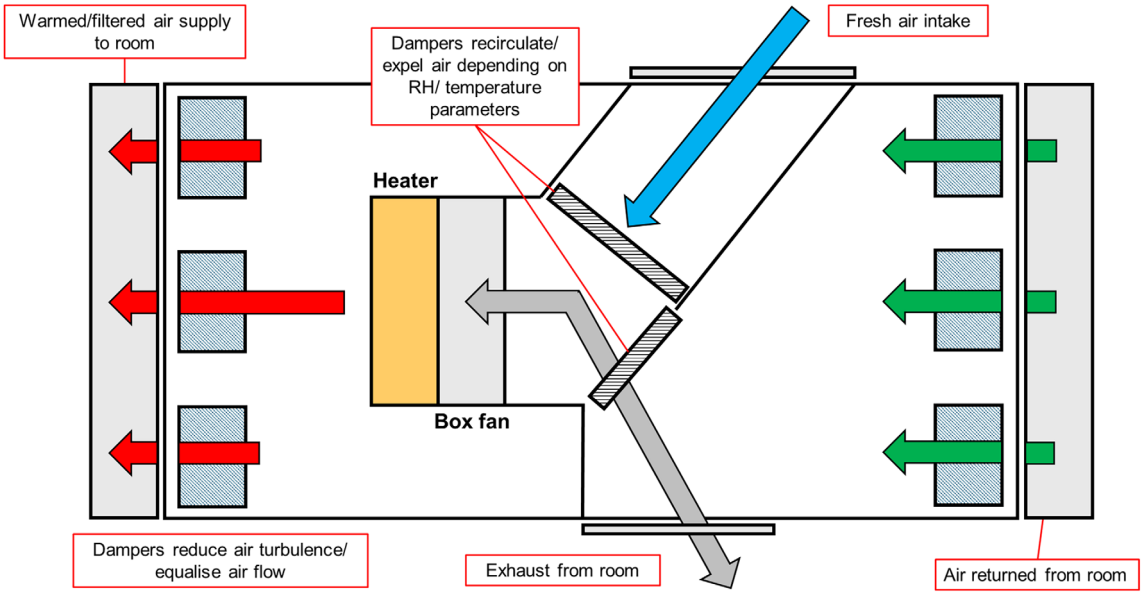

FIGURE 1 A simplified diagram showing the structure and function of the bio-conversion unit (BCU) system used for the rearing of BSFL. Major components are shown as well as the intended flow of air to and from the rearing chamber (grey 'room'). Airflow arrows marked in blue indicate air from outside the system, and grey is for air moving through/being expelled. Green and red air flow arrows represent air moving out of and returning to the rearing chamber, respectively.

humidity (~70%), temperature (27°C) and ventilation rates, even under varying ambient temperatures ranging from −5°C to +35°C. This ensured optimal conditions for the growth and metabolic activity of the BSFL during the bioconversion process.

Origin of BSF eggs

For the experiment, BSF eggs were used, which were purchased from Beta Bugs (https://www.betabugs.uk).

Experimental conditions

Four different substrates were examined during the experiment. For comparison against the organic byproduct BSFL feed groups ('waste' groups), a CF condition was used as a non-waste product experimental diet. For this condition, a CF 'crumb' was combined with wheat bran in a 30/70% DM ratio and was made up to 70% moisture content. CF was used as it was easily accessible and consistently available year-round, and the manufacturers claim the crumb to be nutritionally balanced.

The 'waste' diets consisted of spent brewer grain (BG), surplus fruit/vegetable matter acquired from local supermarkets (OV) and a 1:1 ratio of BG and OV, referred to as OVBG. These 'waste' food conditions had no added water or bran.

Each experimental trial lasted 2 weeks and was repeated in triplicate; in the first week, the larvae were hatched. Five kilos of the CF condition were prepared in an insect breeding tray of 400 × 600 × 145 mm (produced by Beekenkamp Verpakkingen BV—Cat. No: 6205). To the trays, 1 g of BSFL eggs were added and placed into the BCU for 7 days at 27°C. After the initial diet had allowed the hatching of the eggs and progression into neonates, the contents of the week-1 tray were transferred to a second tray containing 10 Kg of the experimental diet, which was prepared fresh before the transfer and then returned to the BCU. The neonates were allowed to grow for another 7 days, after which the tray contents were homogenised. A 250 g sample of each tray was taken for analysis.

Calculation of yield (drying and separating samples)

Two hundred and fifty grams of samples collected from each tray were manually separated, with BSFL and frass placed into separate glass beakers. BSFL larvae were washed in water and then boiled for 10 min in water to devitalise the larvae. The separated components were then dried in an oven at 65°C for 24–48 h or until completely dry. The components were weighed using a scale, and the larval and remaining substrate/frass weight was recorded. The percentage of dry matter made up by the BSFL compared to substrate/frass was then calculated.

Gut sampling and DNA extraction

Gut samples were gathered for each experiment/repeat. Samples were collected at 14 days. >20 larvae were collected for each sample, placed into a 50 mL tube and frozen at −20°C for devitalisation and storage. Once defrosted, the larvae were washed with de-ionised water and then with 70% ethanol to remove potential contaminating

DNA from the surface. Two incisions were made a few millimetres from the anterior and posterior segments and separated. The entire gut was removed using sterile tweezers and transferred to a sterile 1.5 mL tube. Once >0.25 g of gut material had been collected from the larvae, the tube was flash-frozen in liquid nitrogen and kept at −80°C until DNA extraction. For extraction of DNA, samples were thawed and ~ 0.05 g of gut material was extracted using a PureLink™ Microbiome DNA Purification Kit (Invitrogen, Cat No A29790) following manufacturer's guidelines.

Crude protein analysis

Crude protein analysis was done using a Model 440 CHN/O/S Elemental Analyser (produced by Exeter Analytical, Inc.). 0.005 g of previously dried BSFL was ground into a fine powder using a mortar and pestle and then placed inside a pre-weighed tin capsule. The sample containing capsules was put into the analyser, and the nitrogen content was measured via combustion. Combustion products were passed through various filters in the analyser to remove hydrogen and carbon dioxide, leaving only the remaining nitrogen (and inert helium), where the N_2 concentration is measured. Post-combustion, the calculated nitrogen % was multiplied by the nitrogen–protein conversion factor of 6.25 to achieve the crude protein % by weight. This value is based on the Jones factor,[20] with the average nitrogen content of pure protein being 16% (100/16 = 6.25). Jones's factor has been criticised and repurposed as 5.6.[21] However, Jones's factor of 6.25 is the value used by food nutritional composition values (such as those used for the CF); so for consistency, it was used in this study.

To calculate the crude fat content of the BSFL from each feed condition, the lipid extraction method devised by Bligh and Dyer[22] was used with an additional sonication step added. Five g of oven-dried BSFL (previously separated from the frass and substrate) was powdered in a mortar and pestle. This powder was added to 5 mL of 100% chloroform and 10 mL of 100% methanol and placed in a sonicating water bath at ½ amplitude (30 s on, 30 s off) for 30 min. After sonication, 5 mL of chloroform and 5 mL of Milli-Q water were added. The contents were then filtered, under vacuum, through a Whatman 42 filter (Cat No 1442–055). The filter was then ground up with 5 mL of chloroform and filtered through a second Whatman filter. The resulting liquid was allowed to separate. The chloroform layer was removed and placed in a glass vial using a glass pipette. A flow of nitrogen gas was used to evaporate the chloroform. The dried lipids (in the vial) were then weighed and the lipid weight was calculated.

Preparation of samples for bacterial microbiome sequencing

Extracted DNA was sequenced using the Illumina NovaSeq 6000 platform; the V3-V4 region of the prokaryotic 16 s ribosomal subunit was amplified using the 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGGTATCTAAT) primer set. The library underwent MiSeq amplicon sequencing, which Novogene performed with the Illumina NovaSeq 6000 platform, using a paired-end 2 × 250 base pair method. Novogene also performed library preparation and provided reads that were purified and demultiplexed, and had adaptors removed. Raw data are available in NCBI under the PRJNA1174727 project.

Data processing and data analysis

The prepared raw reads supplied by Novogene were analysed with the LotuS2 software.[23] Chimaeras were identified and removed with minimap 2.[24] The Divisive Amplicon Denoising Algorithm 2 (DADA2) was used to cluster reads into ASVs with a dissimilarity of no more than a single nucleotide.[25] Taxonomic classification of clustered ASVs was performed down to the genus level using BLAST[(26)]; ASVs were assigned against a V3–V4 trimmed greengenes2 (GG2) database (version 2022.10).[27] GG2 was selected as it is a chimaera-checked 16 s *rRNA* database, designed for microbiota sequencing data.

Data manipulation, statistical analysis and figure production were performed within Rstudio (version 4.2.3). Data was first rarified to a sequencing depth of 50,000 reads to avoid errors caused by uneven sequencing (this resulted in the loss of 90 ASVS, but preserved low-depth samples). Rarefaction was chosen after consulting rarefaction curves (Supplementary Figure S1) as some samples were shown to have a lower sequencing depth while species count had plateaued prior to 50,000 reads. From the rarefied data, diversity metrics were analysed using Shannon, Chao1, Simpson and true richness (observed taxa). Statistical analysis between diversity metric values was performed using ANOVA/Kruskal–Wallis tests (dependent on data normality, determined by Shapiro–Wilk test), post-hoc Dunn or Tukey HSD pairwise comparisons were then conducted. Principal coordinate analysis (PCoA) was performed to compare microbiota composition utilising PERMANOVA to determine statistically significant distances between sample groups.[28,29]

Micronutrient extraction and analysis using atomic emission spectroscopy

The micronutrient content of the BSFL was calculated using atomic emission spectroscopy (AES). The protocol for this extraction was similar to that reported in.[30] To summarise, eight nutritionally relevant micronutrients were monitored in the BSFL: Na, Ca, K, Mg, Cu, Fe, Mn and Zn. To extract these elements, 0.1 g of previously dried BSFL underwent digestion with 5 mL of concentrated (69%) nitric acid and was heated until boiling using a hotplate. When a white precipitate was observed, the heating was stopped, and the sample was allowed to cool to room temperature. Additional digestion was then performed using hydrochloric acid, and the sample was again heated until a colour change from green to orange was observed. Ten minutes of further heating was then performed. The sample was then made up to

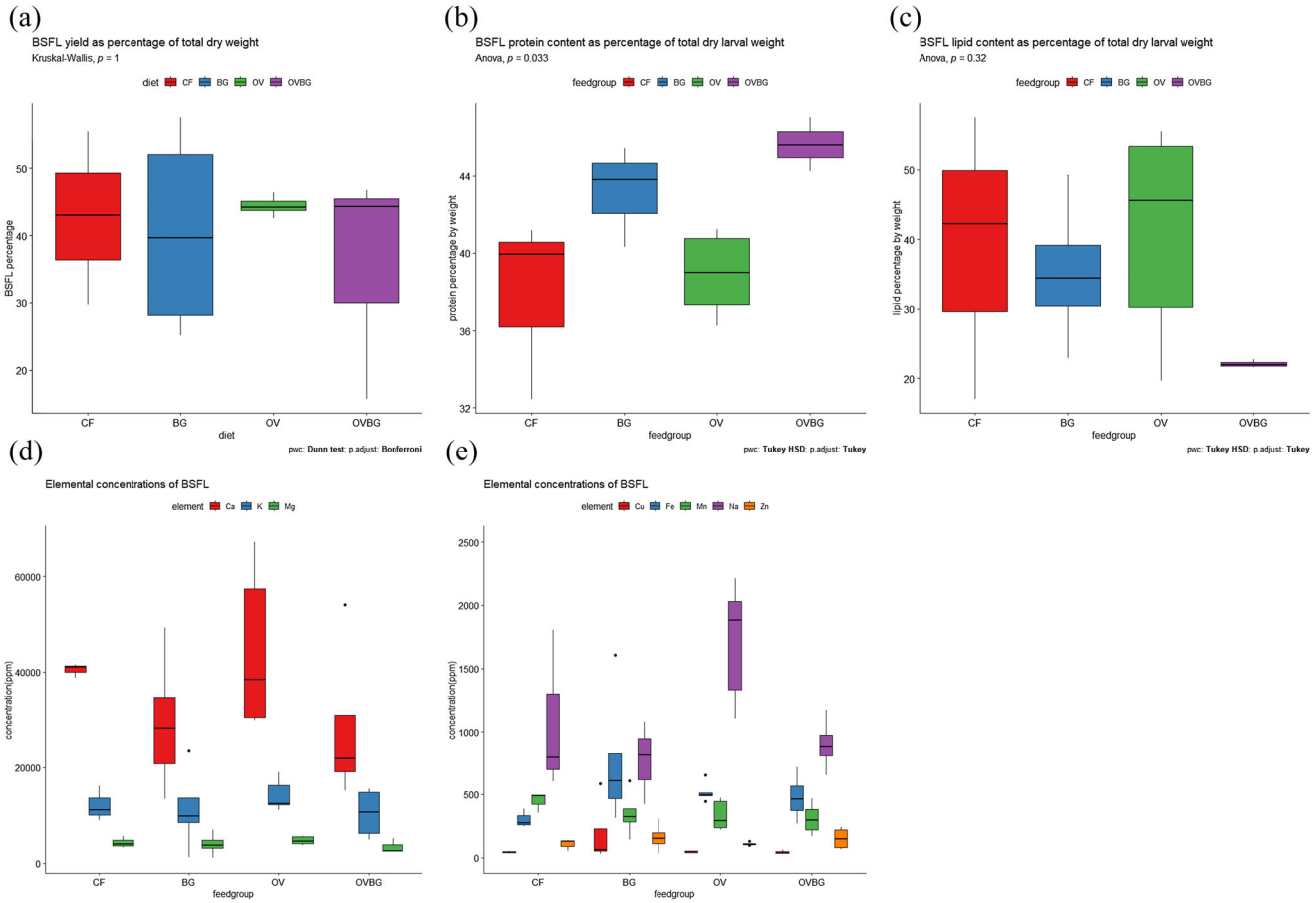

FIGURE 2 Boxplots showing the yield, macro and micronutrient content of the BSFL produced in the BCU utilising different feed conditions/feed groups, (CF- chicken feed, BG- spent brewers grain, OV, waste vegetable/fruit matter, OVBG, combination of BG and OV conditions). Statistical analysis was conducted on the macronutrient contents (a–c) of the samples. Either Kruskal–Wallis H-test and Dunn's test (with Bonferroni p-adjust method) or the ANOVA test and pairwise comparison (Tukey test) were used (based on the data's Shapiro score). Statistical test scores >0.05 indicate significant changes between the samples. (a). Boxplot showing the average yield of BSFL as a percentage of the total weight of each dried sample collected from each feed group. (b). Boxplot showing the average crude protein content of BSFL collected from each feed group. The protein content is shown as a percentage of the total mass of dried BSFL collected at each sample (calculated from nitrogen content and Jones' factor). (c). Boxplot showing the average crude lipid content of BSFL from each feed group. The lipid content is shown as a percentage by weight of the dried BSFL collected from each sample.

100 mL, the extraction was then filtered and stored until analysis by AES.

AES was conducted using an Agilent 4210 MP-AES spectroscope (Cat 4210 MP-AES). Calibration curves were produced using elemental standards dissolved in a 5% HNO_3 and 3% HCL solution at 0.5, 1, 2, 5, 10, 20 ppm. As 0.1 g of dried BSFL was made up to a final volume of 100 mL in the extraction process, the resulting PPM of each element was multiplied by a factor of 1000 to convert PPM to mg/Kg.

RESULTS

To test the functionality of the BCU as a larger-scale BSFL rearing and experimentation device, we first measured the essential characteristics of the BSFLs produced in the experiment. The characteristics of interest are the dry mass of larvae, the total protein and the fat content. Monitoring these characteristics would inform us whether the BCU or the experimental diets resulted in significant changes in larval suitability as a feedstock.

The first of the characteristics of interest studied was each feed group's BSFL yield. This was measured as the % of the total dry mass of each 250 g sample corresponding to BSFL weight (Figure 2a). The four feed groups showed similar average BSFL yields, varying between a low of 35.5% (OVBG) and a high of 44.4% (OV) by dry weight. There was A significant variation between the yield values of each sample within each feed group (with the exception of the OV group, which showed similar yields across all samples). Statistical analysis via the Kruskal–Wallis test, a non-parametric alternative to one-way ANOVA, was chosen as the Shapiro test of the data showed a non-parametric distribution (Shapiro–Wilkes test *p*-value: 0.057). The post-hoc Dunn's test, combined with Kruskal–Wallis, showed no significant differences between the average yield of each feed-group condition.

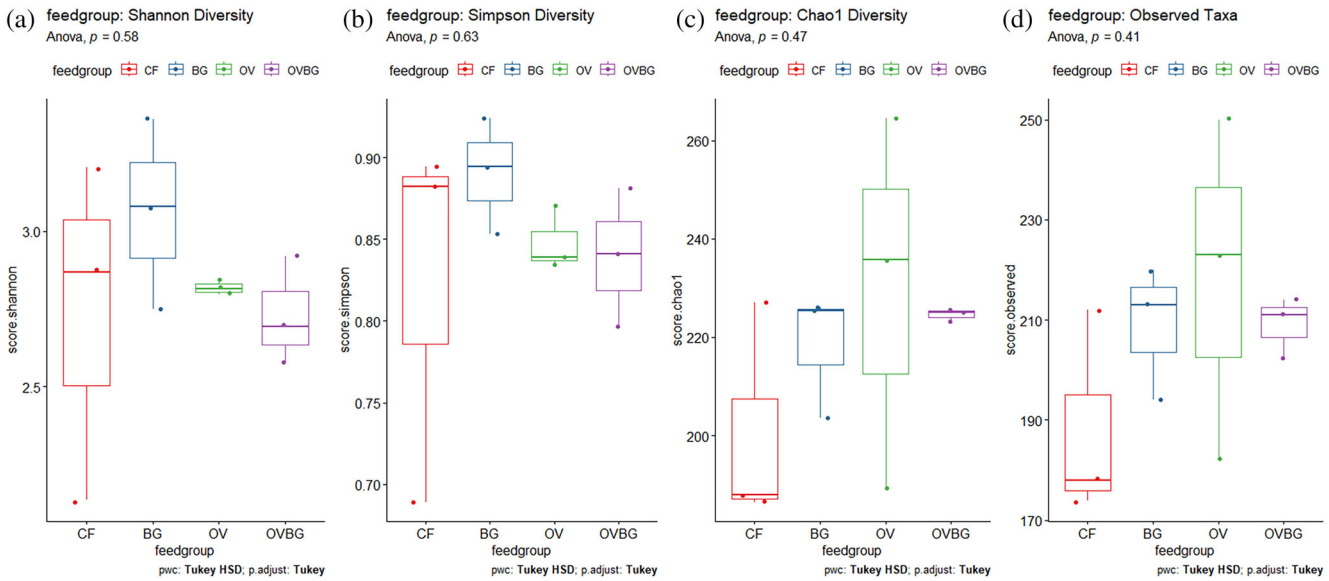

FIGURE 3 Boxplots showing the changes in diversity of samples collected from larvae trays (AGRI, larvae from the AgriGrub supplier; CF, chicken feed; BG, Brewers spent grain; OV, vegetable waste; OVBG, combination of BG and OV conditions) measured using various diversity matrices; Shannon, Chao1 and Simpson. Statistical analysis of the samples, for samples with a normal distribution, the ANOVA test and Tukey test were used. The Kruskal–Wallis and ANOVA scores are all shown to be >0.05, indicating no significant difference between the samples.

Having shown that the dry matter yield of larvae was consistent on waste products, the macronutrient content of the larvae was analysed. Larval protein content was calculated using a mass analyser, and the nitrogen content of the BSFL was used to estimate crude protein content (Figure 2b). All the food conditions for waste had similar or improved protein content to that of the control group (BG: 43.2%, OV: 38.9%, OVBG: 45.7% and CF: 37.9%). Larvae from the OVBG groups significantly outperformed CF with an average crude protein content of >45%. Statistical analysis of crude protein content was performed using ANOVA (as the Shapiro–Wilkes showed a normal distribution). Pairwise analysis (Tukey) found no significance between any two groups.

Having shown that the protein content of BCU-reared larvae was statistically consistent across all feed groups, the crude lipid content was measured (Figure 2c). Analysis showed notable variation in each feed group's crude lipid content. CF (39.0%) and OV (35.2%) had the highest crude lipid content by mass. OVBG performed very poorly in this regard, with an average percentage lipid by weight of 22.1%. Despite these differences in the crude lipid content, statistical analysis (ANOVA, Tukey for pairwise comparison) showed no significant differences between the feed groups.

The macronutrient composition of larvae reared in the BCU on waste food was found to be comparable to that of larvae raised on a nutritionally balanced diet. However, for these larvae to be considered a viable supplementary feedstock for poultry, the micronutrient profile also needed to be assessed to ensure it met the nutritional requirements necessary for chicken health and development. The resulting nutrient content of each element of interest is shown in Figure 2d,e, showing the average PPM of the studied micronutrients were reasonably consistent across the feed groups. All samples showed a very high calcium concentration, with the average calcium PPM for each condition varying between 20,000–45,000 PPM, with CF (40,459.2 PPM) and OV (44,713.7 PPM) feed groups having the highest average Ca content. The waste diets (OV, OVBG and BG) showed considerably more variation in their Ca content. Cu, Fe, Mn, Na and Zn also showed considerable variation in each feed group (Figure 2d), with the waste-food conditions having similarly low PPM of copper and zinc compared to that of CF-reared BSFL. Iron and Manganese fluctuated across the groups, with CF-reared BSFL containing the least iron (303.3 PPM).

This study aimed to support the development of a healthy gut microbiome in the BSFL, as a well-balanced microbiome is a crucial indicator of overall organism health and vitality. To investigate this, the DNA extracted from the gut of the larvae was sent for high-throughput amplicon sequencing. Four diversity metrics (Shannon, Simpson, Chao1 and observed taxa) were used to capture as much information about the BSFL microbiome as possible (Figure 3). ANOVA was used to compare statistical differences between the average diversity scores (Shapiro–Wilkes *p*-value scores were >0.05 for all metrics, implying a normal distribution of values). The Shannon index values (Figure 3a), as indicated by an ANOVA P-value of >0.05 (*p*-value 0.81), had no significant differences between the Shannon scores of each feed group. The average Shannon score for each group exceeded 2.5, implying a diverse and rich microbiota (as Shannon is strongly affected by richness and the presence of rare taxa). The lack of statistical difference indicates all feed-groups had a similarly rich microbiota. The Simpson index (Figure 3b) was used to measure dominance, indicating whether a small number of taxa comprise most of the sequenced reads. There was considerable variation within samples of the CF feed group; however, all feed-groups had an average

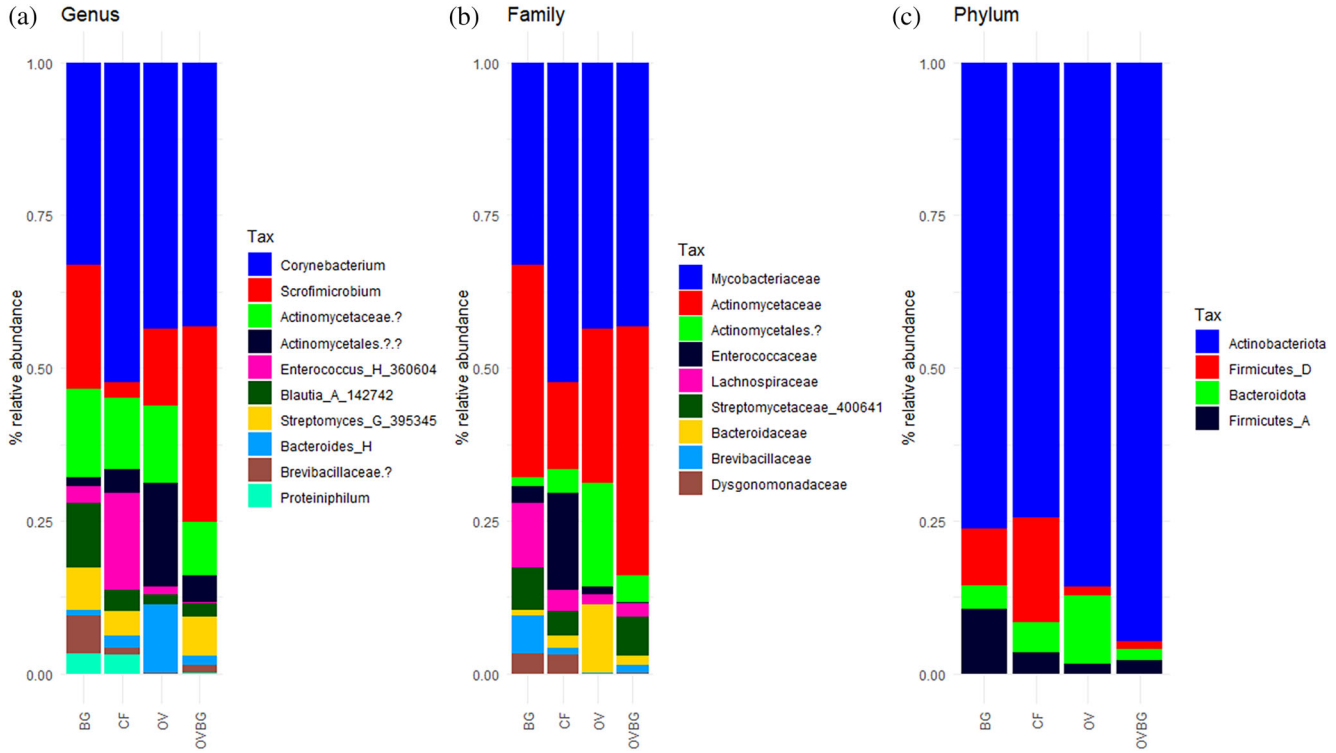

FIGURE 4 Composition plots showing the relative abundances of genus, family and phylum level taxa within the extracted gut DNA from BSFL harvested after 7 days on control diets, samples from each feed group (AGRI, lab-grown BSFL from AgriGrub; CF, chicken feed; BG, Brewers spent grain; OV, vegetable waste; OVBG- combination of BG and OV conditions) have been averaged. All taxa that made up <1% of total abundances have been removed from the plot. The absolute prevalence values of each taxon are shown in Supplementary Table S1.

Simpson score exceeding 0.84, supporting the Shannon index and implying low dominance of the samples by a few taxa and the presence of a rich microbiota. The ANOVA *p*-value of 0.63 indicates that there is no significant difference in the state of microbiota dominance between each feed group. The Chao1 index was used in tandem to highlight the presence of rare taxa (singletons and doubletons) present in the BSFL microbiota (Figure 3c). ANOVA test shows that there are not statistically significant differences between the average Chao1 score of each feed-group (*p*-value 0.47). The waste conditions (BG, OV, OVBG) present a higher Chao1 score than CF. These higher scores indicate more rare taxa within the waste conditions than the CF control, potentially implying richer taxa diversity. True richness, the number of unique taxa observed, was also monitored. The observed average taxa number (Figure 3d) was similar across all experimental substrates, with an ANOVA *p*-value of 0.41, indicating no significant change in true richness. Almost identical results were seen to the Chao1 values, a similar substantial variation was observed within the triplicate samples of the OV feed group.

Having monitored the overall diversity scores of the BSFL microbiome in each feed group, a range of taxonomic compositional plots were produced to visualise the differences observed in Figure 3. The compositional plots indicate the genus, family and phylum level taxa whose average relative abundance comprised >1% of the reads observed in each DNA sample; the relative abundance of each taxon has then been averaged per feed group (Figure 4). Plots showing sample-specific abundances were also produced (Supplementary Figure S2). Across all genus level feed groups, *Corynebacterium* represented the dominant genus in terms of relative abundance, with *Scrofmicrobium* and an unclassified member of the family *Actinomycetaceae* also contributing to the most abundant taxa observed. This trend of the taxa exceeding 1% relative abundance being dominated by a few members is shown at family and Phylum level. *Mycobacteriaceae* and *Actinomycetaceae* dominate the family level plots (Figure 4b). Overall, there is little difference observed between the microbiota from samples BG, CF and OV. Notably, the OVBG feed group showed a considerably higher average relative abundance of *Scrofmicrobium*. The observation that a handful of taxa made up a large portion of all samples necessitated the need for profiling of the core taxa (Supplementary Figure S2). Therein are all taxa present in at least 50% of all samples; *Corynebacterium* was the only taxon present in 100% of samples, making up ≥20% of the relative abundance. This is closely followed by the unclassified member of the family *Actinomycetaceae* (making up at least 5% of the relative abundance in 90% of samples) and *Scrofmicrobium* (making up ≥20% of the relative abundance in ~40% of samples) (Supplementary Figure S2). PCoA (Figure 5) was utilised to show the relationship between the overall microbiota composition (as compositional plots capture only the most abundant taxa). Shown in Figure 5 are the Bray–Curtis dissimilarity matrix distance values of all the samples in the study. Between the two MDS (multidimensional scaling) axes, 47.4% of the total difference between

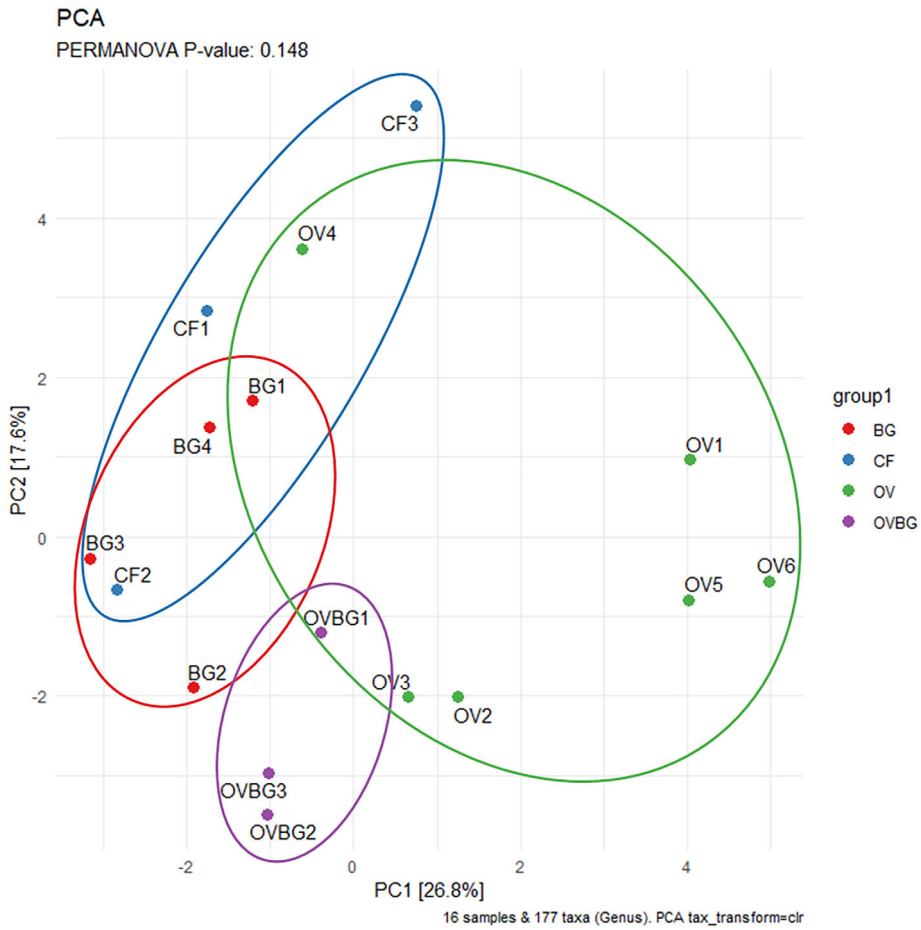

FIGURE 5 Bray–Curtis distance-based principal coordinate analysis (PCoA) plot showing the multidimensional scaling ordination of the samples at genus level aggregation. Samples from the same feed group are shown sharing the same symbol/colour (CF, chicken feed, BG, Brewers spent grain, OV, vegetable waste, OVBG, combination of BG and OV conditions). PERMANOVA analysis was performed on the samples' Bray–Curtis distances (at genus level) when grouped by feed group to determine if there are significant differences between the positions of the groups; the *p*-value, R-squared and F-value are shown at the top of the plot.

samples was captured, with the remaining difference between microbiota likely owing to the unavoidable variance between replicates in vegetable substrate, tray position within the BCU and general noise. To some extent, the samples are distinguishable in their separate feed groups, with the OVBG group occupying the right-hand side of the PCoA plot and being closer to the OV group. The clustering of BG, OV and OVBG samples on the right of the plot possibly implies that there are underlying similarities in the microbiota of BSFL-fed waste substrates, and the grouping was of interest. To determine if the positions of these groups were significantly separated, the permutational multivariate analysis of variance (PERMANOVA) method was used, conducted on the sample data (grouped by feed group and aggregated at genus level). The PERMANOVA null hypothesis implies that the centroids and dispersion of each set of samples (defined by the feed group) will be equidistant. The resultant PERMANOVA *p*-value score of 0.144 accepts the null hypothesis, determining that the samples (when grouped by feed group) do not have an uneven distribution and are not significantly distant from each other. Therefore, despite the visual differences between the groups (microbiota content), the distance matrix values of each feed group are statistically similar. However, it should be noted that PERMANOVA is prone to type II errors (false negatives) when the sample count is low, as there are only three samples within each group, the statistical power of this test is not as strong as it otherwise normally would be. Additionally, it should be noted that PERMANOVA outcomes are sensitive to both centroid location and group dispersion, and caution is required in interpreting non-significant results, particularly with low sample sizes.

DISCUSSION

The results of this study highlight the efficacy and versatility of the novel BCU for larger-scale BSFL production, waste valorisation and scientific research. Our findings suggest that the BCU provides a reliable system for rearing BSFL as a sustainable feed additive and offers a scalable platform for microbiome research that addresses the limitations typically associated with laboratory-scale experiments.

TABLE 1 Table showing the micro (PPM/mg/Kg) and macro (% by weight) nutrient content of dried BSFL compared against the nutrient content of the chicken feed used as a control in the experiment.

	Dried BSFL elemental concentrations (PPM/mg/kg)				
Element	**CF**	**BG**	**OV**	**OVBG**	**Chicken feed concentration(PPM/mg/kg)**
Ca	40459.2	29302.4	44713.7	28259.3	730
Mg	4338.2	3995.4	4745.5	3827.2	6110
Na	1067.3	1017.0	1712.3	897.4	20
K	12096.4	11391.1	14219.6	10454.0	11,820
Fe	303.3	765.9	519.3	478.4	105.7
Zn	107.2	160.1	108.0	151.0	72.7
Mn	446.7	348.9	333.6	307.4	115
Cu	44.4	191.9	45.8	43.6	10

	Dried BSFL elemental concentrations (% by weight)					
	CF	**BG**	**OV**	**OVBG**	**Chicken Feed (% by weight)**	**Bran (% by weight)**
Crude protein	37.9	43.2	38.9	45.7	16.0	15.6
Crude Lipids	39.0	35.2	41.3	22.1	4.0	4.3

The BCU was explicitly designed to offer consistent environmental control over temperature, humidity and ventilation, allowing it to mitigate the variability often introduced by external conditions. This ability to tightly regulate environmental factors is particularly significant in industrial-scale production, where maintaining optimal conditions across large volumes of substrate and larvae can be challenging. The consistent yields and nutrient profiles of the BSFL across various substrates reflect the BCU's capacity to provide stable and reproducible conditions, which are critical for both commercial and research applications.

One of the most promising findings from this study is the ability of the BCU to produce larvae with comparable or superior macronutrient profiles to regular CF (Table 1), particularly in protein and fat content when fed on various waste substrates. This aligns with the growing need for alternative protein sources in livestock feed as global demand for animal products continues to rise. The BSFL produced in the BCU meets the nutritional content of Poultry feed, with higher levels of every measured macro and micronutrient (except for magnesium). The BCU produces this feed additive reliably and does so with a fraction of the environmental cost associated with traditional feedstocks like soy and fishmeal.[31] Given that soy production is linked to significant environmental degradation, such as deforestation and habitat loss,[10] adopting BSFL as a protein-rich feed supplement could help reduce livestock production's environmental impact while providing a reliable, cost-effective alternative.

Furthermore, our micronutrient analysis showed that BSFL reared in the BCU was particularly rich in calcium, with concentrations far exceeding those found in conventional poultry feed. This enhanced calcium content, combined with the larvae's high levels of essential micronutrients like potassium and iron, makes BSFL a highly nutritious feed option that could potentially improve animal health, bone strength and overall growth performance. These benefits, coupled with the larvae's capacity to convert waste materials into valuable biomass, underscore the BCU's potential as an essential component of sustainable agriculture and circular bioeconomy practices.

From a research perspective, the BCU proves to be an invaluable tool for advancing research on BSFL at an industrial scale. One of the key outcomes of this study was the successful replication of microbiota findings observed at the laboratory scale, with some notable additions. The consistent presence of *Corynebacterium*, *Scrofmicrobium* and an unclassified *Actinomycetaceae* in all feed groups (making up at least 5% of the total relative abundance in >50% of samples [Supplementary Figure S3]) and *Corynebacterium*, *Blautia* and an unclassified *Actinomycetaceae* making up at least 1% of the total relative abundance in >80% of samples (Supplementary Figure S4) suggests the presence of a stable core microbiota across various and nutritionally variable substrates; however, relative abundance is not quantitative, so this cannot be said to be definitive. The environmental stability of the BCU is essential for understanding the role of this core microbiota in BSFL health and development and its ability to break down complex organic materials. However, the emergence of *Corynebacterium* as a dominant genus, in contrast to previous lab-scale studies,[18,32] highlights the influence of larger-scale BSFL production conditions on microbiota composition and underscores the importance of conducting larger-scale experiments alongside lab experiments to capture these variations.

The presence of *Corynebacterium* and *Actinomyces* within the BSFL microbiota, genera known for their roles in organic matter decomposition (composting) and nutrient cycling,[33] supports the hypothesis that the BSFL microbiome plays an active role in enhancing the larvae's ability to thrive on diverse, nutrient-poor substrates. This suggests that the microbiota not only contributes to the larvae's resilience but also enhances their capacity to convert waste products into valuable protein and fat-rich biomass. It has been demonstrated in vitro that BSFL possess antimicrobial properties, partially attributed to their microbiota and the production of antimicrobial peptides

(AMPS),[34] further reinforcing the suitability of BSFL as a sustainable feedstock that can be produced on normally pathogenic substrates. It is important to highlight that the microbiome findings presented here are exploratory and should be interpreted as hypothesis-generating due to the small sample size ($n = 3$ per group). The limited statistical power means that although some trends are visually apparent, definitive conclusions cannot be drawn without further validation through larger-scale studies.

In addition to its commercial applications, the BCU also holds promise as a platform for further scientific exploration. For example, future research could investigate the potential of BSFL frass (the organic byproduct of larvae digestion) as a soil amendment or fertiliser. Given the high microbial diversity present in BSFL frass,[35,36] there is potential for its application in sustainable agriculture as a biofertiliser that not only adds nutrients to the soil but also introduces beneficial microorganisms that could enhance plant growth and soil health. Studies on frass have shown promising results in improving the growth of seedlings in the soil,[37] which could make the BCU a dual-purpose system for feed and fertiliser production. The successful implementation of the BCU also demonstrates the scalability of BSFL production for use in industrial applications. As this study has shown, the BCU can be deployed in various settings and fed with locally sourced organic byproducts (waste) materials, making it adaptable to different regional contexts and reducing the need to transport bulky animal feedstocks. This modularity and scalability make the BCU an attractive option for both small and large-scale farming operations, particularly in regions where access to traditional feedstocks is limited or costly.

In conclusion, the BCU represents a significant advancement in the sustainable production of BSFL for both commercial and research purposes. Its ability to maintain consistent yields and nutrient profiles across diverse substrates, coupled with its robust environmental controls, positions the BCU as a reliable and scalable solution for large-scale BSFL production. Furthermore, the BCU offers a unique platform for microbiome research, bridging the gap between laboratory and industrial scales and providing new insights into the role of the microbiome in waste conversion and nutrient cycling. The versatility and potential applications of the BCU make it a valuable tool for advancing sustainable agriculture and addressing the growing demand for alternative protein sources in animal feed.

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CONFLICT OF INTEREST STATEMENT

Richard Small is the managing director and founder of Inspro-UK, a company focused on improving the valorisation of food waste.

DATA AVAILABILITY STATEMENT

The raw sequencing data generated and analysed during this study are publicly available in the NCBI BioProject database under accession number PRJNA1174727 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1174727).

ETHICS STATEMENT

No ethics statement was required.

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REFERENCES

1. Wang YS, Shelomi M. Review of black soldier Fly (*Hermetia illucens*) as animal feed and human food. Foods. 2017;6(10):91.
2. Kaya C, Generalovic TN, Ståhls G, Hauser M, Samayoa AC, Nunes-Silva CG, et al. Global population genetic structure and demographic trajectories of the black soldier fly, Hermetia illucens. BMC Biol. 2021;19(1):1–22.
3. Lindner P. Zur Fettgewinnung aus Kleintieren [recovery of fat from small animals]. Z Tech Biol. 1919;7:213–20.
4. Banks IJ, Gibson WT, Cameron MM. Growth rates of black soldier fly larvae fed on fresh human faeces and their implication for improving sanitation. Trop Med Int Health. 2014;19(1):14–22.
5. Newton GL, Booram CV, Barker RW, Hale OM. Dried *Hermetia Illucens* larvae meal as a supplement for swine. J Anim Sci. 1977;44(3):395–400.
6. Sheppard DC, Tomberlin JK, Joyce JA, Kiser BC, Sumner SM. Rearing methods for the black soldier fly (Diptera: Stratiomyidae). J Med Entomol. 2002;39(4):695–8.
7. FAO (Food and Agriculture Organisation). Gateway to poultry production and products. 2024.
8. Messina M. Perspective: soybeans can help address the caloric and protein needs of a growing global population. Front Nutr. 2022;9:909464.
9. EU. The European Comission: The Commission recognises the U.S. Soya bean – scheme as compatible with EU sustainability standards. 2019.
10. Sauer S. Soy expansion into the agricultural frontiers of the Brazilian Amazon: the agribusiness economy and its social and environmental conflicts. Land Use Policy. 2018;79:326–38.
11. Sealey WM, Gaylord TG, Barrows FT, Tomberlin JK, McGuire MA, Ross C, et al. Sensory analysis of rainbow trout, Oncorhynchus mykiss, fed enriched black soldier Fly prepupae, *Hermetia illucens*. J World Aquacult Soc. 2011;42(1):34–45.
12. Cullere M, Tasoniero G, Giaccone V, Miotti-Scapin R, Claeys E, de Smet S, et al. Black soldier fly as dietary protein source for broiler quails: apparent digestibility, excreta microbial load, feed choice, performance, carcass and meat traits. Animal. 2016;10(12):1923–30.
13. Schiavone A, de Marco M, Martínez S, Dabbou S, Renna M, Madrid J, et al. Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (*Hermetia illucens* L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. J Anim Sci Biotechnol. 2017;8(1):1–9.
14. Nampijja Z, Kiggundu M, Kigozi A, Lugya A, Magala H, Ssepuuya G, et al. Optimal substitution of black soldier fly larvae for fish in broiler chicken diets. Sci Afr. 2023;20:e01636.

15. EU. European Comission: amending Annexes I and IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council and Annexes X, XIV and XV to Commission Regulation (EU) No 142/2011 as regards the provisions on processed animal protein. 2017.
16. Barragan-Fonseca KB, Dicke M, van Loon JJA. Influence of larval density and dietary nutrient concentration on performance, body protein, and fat contents of black soldier fly larvae (*Hermetia illucens*). Entomol Exp Appl. 2018;166(9):761–70.
17. Deruytter D, Gasco L, Yakti W, Katz H, Coudron CL, Gligorescu A, et al. Standardising black soldier fly larvae feeding experiments: an initial protocol and variability estimates. J Insects Food Feed. 2023;1:1–12.
18. Klammsteiner T, Walter A, Bogataj T, Heussler CD, Stres B, Steiner FM, et al. The Core gut microbiome of black soldier Fly (*Hermetia illucens*) larvae raised on low-bioburden diets. Front Microbiol. 2020;11:499002.
19. Miranda CD, Cammack JA, Tomberlin JK. Mass production of the black soldier Fly, *Hermetia illucens* (L.), (Diptera: Stratiomyidae) reared on three manure types. Animals. 2020;10(7):1–11.
20. Jones DB. Factors for converting percentages of nitrogen in foods and feeds into percentages of protein. Washington, DC: US Department of Agriculture; 1941. p. 183.
21. Mariotti F, Tomé D, Patureau Mirand P, Ois Mariotti F, Tométom D. Converting nitrogen into protein—beyond 6.25 and Jones' factors. Crit Rev Food Sci Nutr. 2008;48(2):177–84.
22. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol. 1959;37(8):911–7.
23. Özkurt E, Fritscher J, Soranzo N, Ng DYK, Davey RP, Bahram M, et al. LotuS2: an ultrafast and highly accurate tool for amplicon sequencing analysis. Microbiome. 2022;10(1):1–14.
24. Li H. Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics. 2018;34(18):3094–100. https://doi.org/10.1093/bioinformatics/bty191.
25. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13(7):581–3.
26. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215(3):403–10.
27. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol. 2006;72(7):5069.
28. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12(6):1–18.
29. Anderson MJ. Permutational Multivariate Analysis of Variance (PERMANOVA). Wiley Stats Ref Stat Ref. 2017;1–15. https://doi.org/10.1002/9781118445112.stat07841.
30. Zulkifli NFNM, Seok-Kian AY, Seng LL, Mustafa S, Kim YS, Shapawi R. Nutritional value of black soldier fly (*Hermetia illucens*) larvae processed by different methods. PLoS One. 2022;17(2):e0263924.
31. Nunes AJP, Dalen LL, Leonardi G, Burri L. Developing sustainable, cost-effective and high-performance shrimp feed formulations containing low fish meal levels. Aquac Rep. 2022;27:101422.
32. IJdema F, de Smet J, Crauwels S, Lievens B, van Campenhout L. Meta-analysis of larvae of the black soldier fly (*Hermetia illucens*) microbiota based on 16S rRNA gene amplicon sequencing. FEMS Microbiol Ecol. 2022;98(9):1–13.
33. Franke-Whittle IH, Knapp BA, Fuchs J, Kaufmann R, Insam H. Application of COMPOCHIP microarray to investigate the bacterial communities of different composts. Microb Ecol. 2009;57(3):510–21.
34. Vogel H, Müller A, Heckel DG, Gutzeit H, Vilcinskas A. Nutritional immunology: diversification and diet-dependent expression of antimicrobial peptides in the black soldier fly *Hermetia illucens*. Dev Comp Immunol. 2018;78:141–8.
35. Basri NEA, Azman NA, Ahmad IK, Suja F, Jalil NAA, Amrul NF. Potential applications of Frass derived from black soldier Fly larvae treatment of food waste: a review. Foods. 2022;11(17):2664.
36. Schmitt E, de Vries W. Potential benefits of using *Hermetia illucens* frass as a soil amendment on food production and for environmental impact reduction. Curr Opin Green Sustain Chem. 2020;25:100335.
37. Carroll A, Fitzpatrick M, Hodge S. The effects of two organic soil amendments, biochar and insect Frass fertilizer, on shoot growth of cereal seedlings. Plants (Basel). 2023;12(5):1071.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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