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Enhancing sustainability in meat production through insect biorefinery



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Valuable feed crops and fossil fuel energy are used to produce animal meat. To become sustainable, meat production methods must adapt to include non-food substrates and renewable fossil-fuel alternatives. We evaluated the potential of protein livestock feed and biodiesel production through insect biorefining. The bioconversion efficiency of organic waste into black soldier fly larvae (BSFL) biomass was 32.0–35.8% after 24 d. The protein and lipid composition of BSFL changed with the cultivation time. The substrate influenced lipid content, and low lipid content led to lower lipid accumulation in the BSFL. Nevertheless, the potential productivity of proteins (42,471–48,345 kg ha⁻¹ y⁻¹) and lipids (41,642–64,708 kg ha⁻¹ y⁻¹) from BSFL cultivation with organic waste was higher than that of conventional livestock feed/biodiesel feedstocks, such as maize or soybean. In conclusion, insect biorefineries using BSFL can contribute significantly to the establishment of sustainable meat production.

Meat is an essential nutritional and energy source in the human diet because it provides proteins, vitamins, and vital microelements, such as iron and zinc¹. Globally, meat contributes 11%, 21%, and 29% of the daily energy, protein, and fat intake, respectively². The increasing demand for meat, particularly in Asian regions such as China, is attributed to population expansion and increased income³. However, this surge in demand entails increased consumption of meat production-related resources, such as energy and livestock feed⁴. Overusing these resources poses environmental threats, particularly greenhouse gas (GHG) emissions⁵.

Fossil fuels are the primary energy resources driving the contemporary meat industry⁵. Meat production requires massive quantities of fossil fuels, with a six times higher consumption rate to produce alternative protein resources⁶. The heavy reliance on fossil fuels contributes considerably to GHG emissions and exacerbates global warming⁷. Moreover, given that certain livestock feeds are shared with human food consumption, using these food resources for livestock feeds triggers competition and increases resource costs^{8,9}. Note that more than half of harvested maize is allocated to livestock feed, and only 12.8 wt.% of maize is used for human food¹⁰. This competition exacerbates global food insecurity¹¹. Therefore, a sustainable approach to meat production must be developed to address environmental concerns while ensuring food security.

The biorefinery concept represents a sustainable approach to converting biomass into valuable resources such as energy and chemicals¹².

Insect-based biorefineries have emerged as a future circular economy concept owing to their potential for producing biotechnological products¹³. Insect biomass can be used as a direct livestock feed and/or feedstock for fuels/chemicals^{14,15}. Leveraging the omnivorous nature of most insects and feeding them organic (food) waste provides a venue for establishing a circular economic loop that effectively converts waste into valuable resources¹⁶. In particular, black soldier fly larvae (BSFL, *Hermetia illucens*) can digest organic waste and convert it into lipids and proteins^{17,18}. BSFL emits less carbon dioxide (CO₂) than traditional organic waste management platforms using microbes^{19,20}. Furthermore, cultivating BSFL requires less land than other protein resources and can be carried out in arid regions²¹. Their cultivation minimizes competition with agricultural land designated for food production.

Since the lipids and proteins in BSFL biomass are used to produce biodiesel and livestock feed, respectively, monitoring the growth of BSFL and their biochemical profiles is essential to maximize lipid and protein yields. Although numerous studies have been conducted on feeding BSFL with organic waste such as food waste, manure, and sludge, previous studies have focused on analyzing the biochemical contents of harvested biomass at the endpoint of larval growth^{17,22,23}. Thus, a gap remains in systematically confirming the changes in biochemical compositional profiles throughout the different larval developmental stages. Since metabolic profiles in insects can vary significantly during growth and metamorphosis²⁴, addressing this

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gap is of importance. By understanding these changes, it might be able to enhance the efficiency of protein and lipid production in insect cultivation using organic waste, thereby optimizing the process for better resource utilization and sustainability.

This study monitored the biochemical components (lipids/proteins) of BSFL-fed organic waste (collected from animal feed industries) alongside larval growth. Quantitative and qualitative analyses of lipids (particularly fatty acids (FAs)) in BSFL biomass were performed using a direct conversion method because of its high accuracy for lipid analysis^{25,26}. The nitrogen-to-protein conversion factor determined the BSFL biomass protein content²⁷. The lipid and protein contents were monitored over the elapsed cultivation time. To this end, the lipid and protein productivity was compared with those of other feedstocks used for biodiesel and livestock feed production. The results of this study provide insight into the potential economic viability of insect biorefineries for producing biodiesel and livestock feed for meat production.

Results and discussion

BSFL cultivation with organic waste

BSFL were cultivated for 24 days with organic waste collected from pet feed producing factories (i.e., OW1 and OW2). The moisture content of collected OW1 and OW2 was 4%. The ash content of OW2 was 7%, which was slightly higher than OW1 (6%). Given that the growth and metabolism of BSFL (that is, their ratios of carbohydrates, proteins, and lipids) vary with substrate composition, a biochemical analysis of the organic waste was

conducted (Fig. 1). The lipid content of the waste was determined using Soxhlet analysis. The proportion of lipid compounds in OW2 (15%) was higher than that in OW1 (9%), whereas the carbohydrate and protein contents were higher in OW1 than in OW2.

The average fresh weight of BSFL fed with OW1 and OW2 increased continuously and rapidly as the cultivation time increased (Fig. 2A). The patterns of fresh weight increase between OW1 and OW2-fed BSFL were similar. Indeed, on Day 24, the average fresh weight of OW1-fed BSFL was 209.9 mg larva⁻¹, while that of OW2-fed BSFL was 215.8 mg larva⁻¹. The larval development of BSFL is divided into 6 stages: 1st instar to 6th instar (prepupae)²⁸. Through 5 molts, the larvae became larger, and their weight increased²⁹.

On Day 24, the average weights of BSFL (dry basis) fed with OW1 and OW2 were 65.7 and 73.6 mg larva⁻¹, respectively (Fig. 2B). The initial average weight (dry weight) of the larvae (day 0) was 15.9 mg. These results suggest that organic waste was converted into BSFL biomass. This biological concept, known as bioconversion, refers to the ability of organisms to convert organic materials (waste) into valuable resources³⁰. A high bioconversion efficiency indicates minimizing waste generation while maximizing the production of valuable resources. Because bioconversion efficiency is linked to carbon assimilation (fixation) in BSFL biomass, a low bioconversion efficiency implies a higher GHG potential³¹. Therefore, bioconversion efficiency is a key factor directly linked to the sustainability of the process. The bioconversion efficiencies of OW1 and OW2 using BSFL were 32.0% and 35.8%, respectively. The conversion efficiencies of organic waste by BSFL were 0.2 to 31.8%, which were contingent on the types of substrates¹⁷.

To determine the substrate preference of BSFL, three major nutrients (carbohydrates, proteins, and lipids) in the feed residue (after BSFL cultivation) were determined (Fig. 3). It is presumed that a high consumption rate was observed for the high-preference substances. BSFL showed a high consumption rate of all nutrients under OW1 and OW2-feeding conditions. Nevertheless, the lipid consumption rate was higher in BSFL cultivated with OW1 (89.2%) than OW2 (65.4%). Additionally, the lipid consumption rate in BSFL cultivated with OW1 was higher than that with carbohydrate/protein consumption (79.4 and 74.9%, respectively).

This is likely attributable to the low lipid composition of OW1 (Fig. 1). Indeed, the lipid contents in OW1 and OW2 before feeding on BSFL were 34.6 g and 60.6 g, respectively. The amount of lipids in OW1 residue (after BSFL feeding) was 3.7 g, while 21.0 g of lipids was left in OW2 residue. These results indicate that BSFL consumed 30.6 and 39.6 g of lipids under OW1 and OW2 feeding conditions, respectively. Given that the amount of lipids consumed was higher in BSFL cultivated with OW2, the higher lipid consumption rate over other nutrients in BSFL cultivated with OW1 may not indicate the preference of BSFL for lipid compounds. It is plausible that the limited lipid content of OW1 could distort the substrate preferences of BSFL.

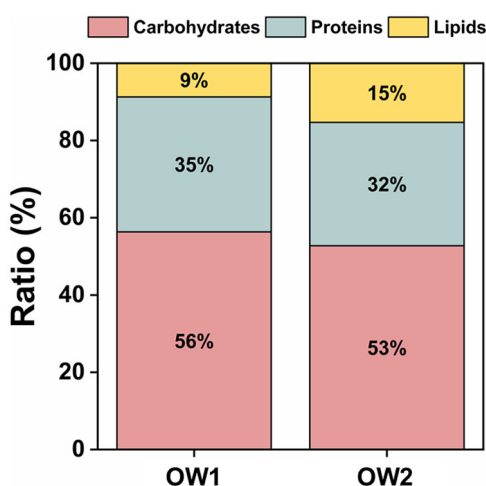


Fig. 1 | Biochemical composition of two organic waste (OW1 and OW2). OW1 and OW2 were collected from pet feed producing factories. The weight of organic waste was on a dry basis. Red, cyan, and yellow colors indicate carbohydrates, proteins, and lipids, respectively.

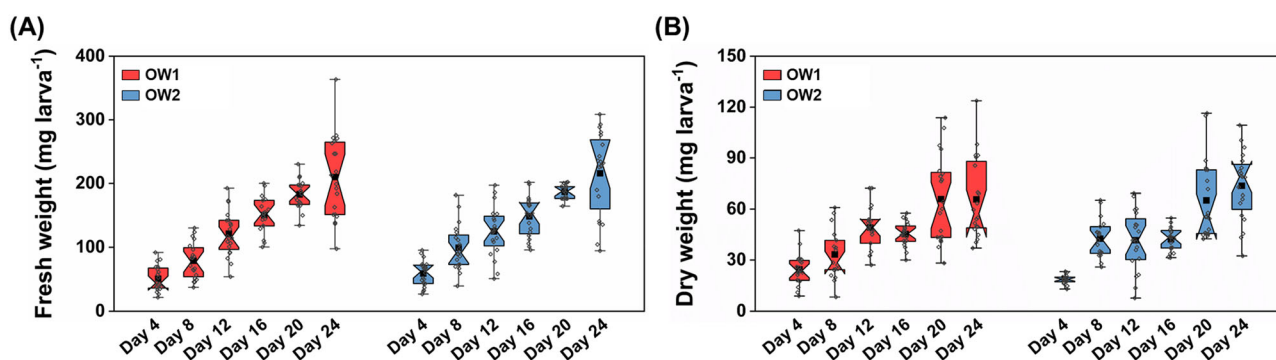


Fig. 2 | Growth of black soldier fly larvae (BSFL)-fed with two organic waste (OW1 and OW2). A Fresh weight of a single larva at each day. B Dry weight of a single larva at each day. ◇ represents individual larval weight and ■ represents the mean.

We hypothesized that the limited lipid compounds in OW1 could affect the growth and/or metabolic activity of BSFL. Indeed, growth patterns based on the dry weight of BSFL differed between BSFL raised on OW1 and OW2. Unlike the trend of the average BSFL fresh weight, which steadily increased, the average BSFL dry weight did not increase continuously (Fig. 2B). In other words, the period when a constant dry weight of BSFL was observed differed according to the type of feed used. The average dry weights of BSFL fed with OW1 on Days 12 and 16 were 49.1 and 45.3 mg larva⁻¹, respectively. The average dry weights of BSFL-fed with OW2 were maintained at 41.7–42.5 mg larva⁻¹ on Days 8–16. The fresh weight of BSFL-fed with OW2 increased continuously during the same period (8: 99.2, 12: 124.9, and 16: 148.6 mg larva⁻¹). These results indicate that the BSFL biomass moisture content changed during cultivation. In short, the period of constant dry weight and increasing fresh weight suggests that the BSFL biomass moisture content increased continuously during that period (Days 12–16 for OW1-fed BSFL and Days 8–16 for OW2-fed BSFL).

Water is essential for living organisms, as it is involved in many biochemical reactions as a solvent and/or reactant³². BSFL-fed, a highly moisturized substrate, reportedly showed higher survival and growth rates³³. The larval feed moisture content is related to their metabolic activities, particularly lipid metabolism³⁴. The BSFL obtained their water from the substrates, and moisture contents of OW1 and OW2 were controlled identically (that is, fresh feeds containing 67 wt.% water). Therefore, it is plausible that high moisture content in the BSFL biomass was associated with metabolic activities. Their moisture content varied due to metabolic event changes during the BSFL development, leading to different fresh/dry weight ratio patterns. To confirm this, biochemical compositional changes in the BSFL biomass were monitored.

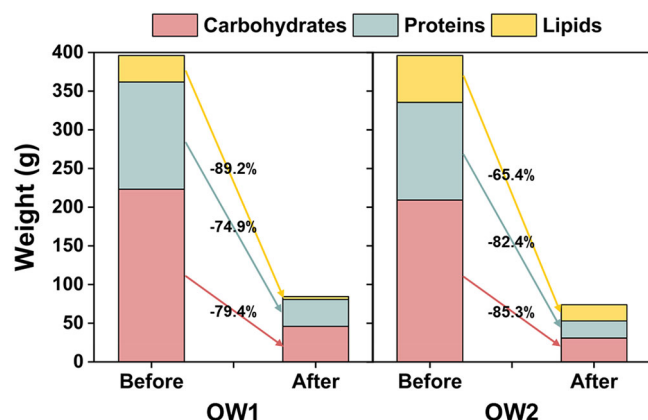


Fig. 3 | Changes in biochemical composition of two organic waste (OW1 and OW2) before/after larvae feeding. Black soldier fly larvae (BSFL) were cultivated for 24 days. The weight of feeds was on a dry basis. Red, cyan, and yellow colors indicate carbohydrates, proteins, and lipids, respectively.

Biochemical properties of BSFL biomass

The BSFL biomass mainly comprises carbohydrates, proteins, lipids, and chitins²⁷. Because the target metabolites for BSFL biomass utilization in this study were proteins and lipids, changes in the biochemical composition of the BSFL were monitored over the elapsed cultivation time, focusing on protein and lipid contents (Fig. 4). The BSFL protein content was determined using a nitrogen-to-protein conversion factor of 4.76. Note that using a general conversion factor (6.25) would overestimate the protein content in BSFL biomass because the biomass contained nitrogen-containing molecules, such as chitin, that are not considered protein²⁷. For the lipid analysis, the direct conversion method was used^{25,26}.

In the BSFL-fed with OW1, the protein content decreased from its initial value to 25.4 wt.% on Day 8, while the lipid content increased from 36.6 wt.% to 44.5 wt.%. However, the lipid content decreased from Days 8 to 24, ending on Day 24 at 29.1 wt.%. Given that the lipid content of OW2-fed BSFL remained stable for 24 days (36.5–40.4 wt.%), the decreased lipid content after Day 8 of OW1-fed BSFL is likely attributed to the limited lipid substrates in OW1 (Fig. 3). These results suggest that lipid accumulation in BSFL was significantly affected by the substrates, particularly their lipid content. BSFL converts carbohydrates into lipids (triglycerides) through a complex metabolic pathway^{35,36}. However, direct uptake of lipids from substrates may be advantageous for lipid accumulation because more energy is required to convert carbohydrates into lipids. These results suggested that including sufficient lipids in BSFL substrates is beneficial when cultivating BSFL to produce lipids (biodiesel).

The biochemical composition of OW1/OW2-fed BSFL on Day 24 was characterized thermogravimetrically (Fig. 5). BSFL-fed with OW1 or OW2 were thermally decomposed at 175 °C and exhibited similar residual mass patterns between the two feeds (Fig. 5A). However, differing rates of thermal decomposition (represented as a differential thermogram (DTG)) between OW1-fed BSFL and OW2-fed BSFL were discernible. The major DTG peaks were observed at 348 and 435 °C in BSFL-fed with OW1 and at 365 and 435 °C in BSFL-fed with OW2 (Fig. 5B). The different DTG patterns indicated that the biochemical composition of the BSFL differed depending on the type of feed. Given that DTG peaks of carbohydrates, proteins, and chitins were observed at ≤400 °C^{37,38}, the major DTG peaks observed at 348 °C (OW2) and 365 °C (OW1) might be attributed to the thermal decomposition of carbohydrates, proteins, and chitins.

Since lipid compounds have higher thermal stability than carbohydrates, proteins, and chitins³⁷, the DTG peaks at 435 °C could originate from the thermal decomposition of BSFL lipids. Notably, the peak at 435 °C of OW2-fed BSFL was larger than that of OW1-fed BSFL. These results support previous results showing that the lipid content in OW2-fed BSFL was higher than in OW1-fed BSFL.

Although the biochemical compositional changes of BSFL consuming different feed types were investigated, the results do not fully explain the constant dry weight periods of the two BSFL cultivations (Days 12–16 for OW1-fed BSFL and 8–16 for OW2-fed BSFL). Given that the main difference between OW1-fed BSFL or OW2-fed BSFL was lipid content, the BSFL lipid metabolism might be key to the different BSFL growth patterns.

Fig. 4 | Changes in protein and lipid contents of black soldier fly larvae (BSFL). BSFL were fed with two organic wastes ((A) OW1 and (B) OW2) for 24 days. Cyan, yellow, and gray colors indicate Proteins, lipids, and others, respectively.

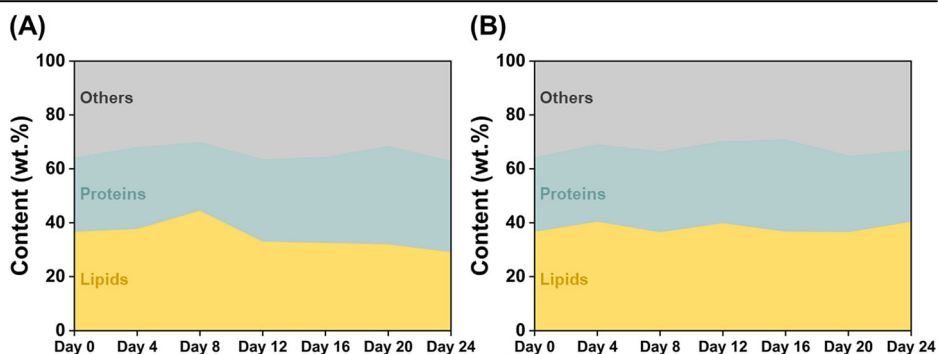


Fig. 5 | Thermal decomposition of black soldier fly larvae (BSFL). A Residual mass. **B** Differential thermogram (DTG) curve. The BSFL were harvested on day 24.

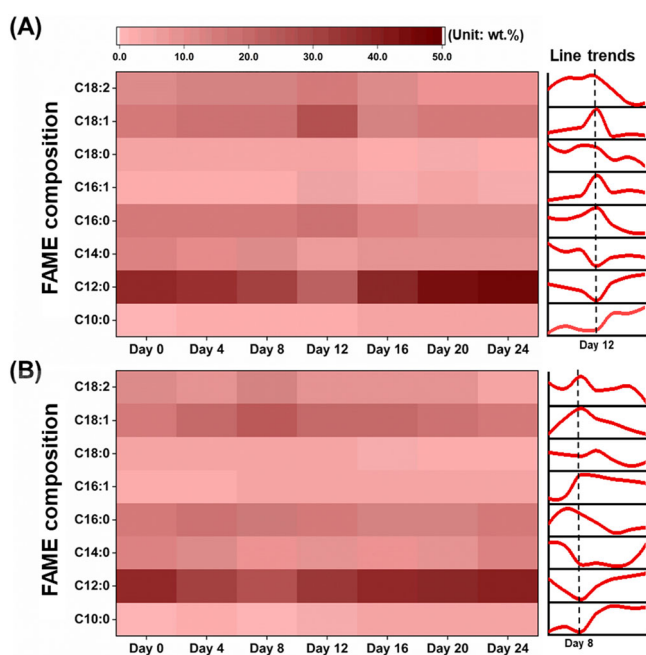
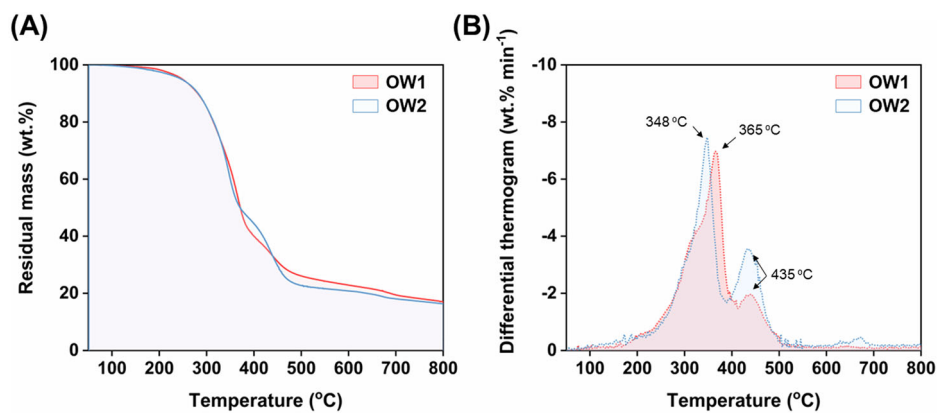


Fig. 6 | Changes in fatty acid (FA) compositions of black soldier fly larvae (BSFL). A FA composition of BSFL-fed with organic waste (OW) 1. **B** FA composition of BSFL-fed with OW 2.

The lipid metabolism can be affected by the BSFL moisture content or vice versa³⁴. Therefore, the FA composition was analyzed to gain insight into the lipid metabolism of BSFL (Fig. 6).

The major BSFL FA components at the beginning of growth (0) were lauric acid (C_{12:0}; 37.3 wt.%), oleic acid (C_{18:1}; 15.6 wt.%), palmitic acid (C_{16:0}; 15.0 wt.%), myristic acid (C_{14:0}; 12.6 wt.%), and linoleic acid (C_{18:2}; 11.3 wt.%). In the cultivation of BSFL fed with OW1, the lauric acid content decreased continuously until its content on Day 12 was found to be 22.3 wt.% (Fig. 6A). The myristic acid content also decreased from 12.6 to 7.1 wt.% during the same period. In contrast, the contents of palmitic acid, palmitoleic acid (C_{16:1}), oleic acid, and linolenic acid increased. On Day 12, oleic acid was the major FA component, accounting for 26.3 wt.% of total FAs.

The FA composition of OW1-fed BSFL changed dramatically from Day 12. Indeed, the content of FAs with carbon numbers ≤14 continuously increased from Day 12 to Day 24. The lauric acid fraction on Day 24 was 46.0 wt.%, 2.1-fold higher than on Day 12 (22.3 wt.%). In contrast, the content of FAs with carbon numbers ≥16 on Day 24 was significantly lower than on Day 12. Palmitic acid, palmitoleic acid, stearic acid (C_{18:0}), oleic acid, and linoleic acid on Day 24 were 11.1, 3.2, 2.0, 14.3, and 7.9 wt.%,

respectively. The palmitic acid, palmitoleic acid, stearic acid, oleic acid, and linoleic acid levels on Day 24 were 36, 43, 47, 46, and 45%, respectively, lower than on Day 12. These results suggest that Day 12 of BSFL cultivation with OW1 may be a turning point in lipid metabolism.

The FA composition trend of OW2-fed BSFL resembled that of BSFL-fed with OW1 in that the content of FAs with carbon number ≥16 increased at the beginning of cultivation. The contents decreased from the turning point (Fig. 6B). In addition, the contents of FAs (carbon number: ≤14) decreased until the turning point and rapidly increased after the turning point. However, the turning point of lipid metabolism differed between larvae-fed with OW1 and OW2. Indeed, the turning point of lipid metabolism in OW1-fed BSFL was Day 12, and in OW2-fed BSFL, Day 8. The turning point of the FA content trend was identical to the initiation point of the constant dry weight period (Fig. 2B). These results suggest that changes in BSFL lipid metabolism were indirectly associated with the moisture content of BSFL biomass.

It has been reported that BSFL accumulates lipids, particularly lauric acid, during the larval development stage to store energy for future utilization³⁹. The chain length of FAs can be shortened through carboxylation and catabolic processes in the gut of BSFL³⁵. Therefore, it is plausible that the BSFL converted the digested lipids (with long chain lengths) into short forms, such as lauric acid and myristic acid. Nevertheless, the reason the turning point of lipid metabolism differs depending on the feed type remains unclear. One possible explanation could be the different established microbiomes in the BSFL guts⁴⁰. For example, the genus *Providencia* plays an essential role in the metabolism of lipids and proteins in BSFL guts⁴¹. Elucidation of the relationship between the gut microbiota and changes in lipid metabolism could be a subject for future research.

Contribution of BSFL-based biorefinery to meat production

Commercial meat production requires many resources, such as live feed and energy⁴². Feed accounts for 70% of the total poultry industry production costs⁴³. A single broiler chicken, for example, consumes 3 kg of feed for growth⁴⁴. In addition, fossil fuels such as diesel are required to produce, feed, and rear broilers⁴⁵, which could hinder sustainable meat production. Therefore, using appropriate biomass (feedstock) for livestock feed and energy production is economically and environmentally beneficial. The lipids in biomass can be converted into liquid fuel (biodiesel) through transesterification⁴⁶. Table 1 summarizes the proteins and lipids produced from different feedstocks.

Soybean and maize produce livestock feed and biodiesel^{10,47}. However, using these feedstocks in livestock farming raises ethical concerns because they are staple crops in numerous communities¹¹. To avoid ethical dilemmas, feedstocks such as jatropha and microalgae that are not used as human food resources have been used for feed and energy production^{46,48}. The high production of proteins and lipids in microalgal cultivation is facilitated by their rapid growth rate and metabolism, which is conducive to increased metabolite accumulation⁴⁹. Nevertheless, the productivities of lipids and

Table 1 | Comparison of protein and lipid productivities from different feedstocks. BSFL indicates black soldier fly larvae and OW represents organic waste

Feedstocks	Biomass productivity (kg ha ⁻¹ y ⁻¹)	Protein		Lipid (oil)		Food vs. feed competition	References
		Content (wt.%)	Productivity (kg ha ⁻¹ y ⁻¹)	Content (wt.%)	Productivity (kg ha ⁻¹ y ⁻¹)		
Jatropha	3542	20.7	733	31.4	1,112	No	48
Maize	7500	4.9	368	9.7	728	Yes	64,65
Soybean	2988	39.3	1,174	20.1	601	Yes	66,67
Microalgae (<i>Chlorella</i>)	41,000	39.0	15,990	26.8	10,988	No	49
Safflower seed	1065	23.0	79	32.1	342	No	68
Mealworm larvae	86,939	68.1	10,302	17.4	15,127	No	50
BSFL-fed with OW1	143,253	33.7	48,345	29.1	41,642	No	This study
BSFL-fed with OW2	160,478	33.2	42,471	40.3	64,708	No	This study

Table 2 | Amino acid compositions of black soldier fly larvae (BSFL) fed with two organic waste (OW1 and OW2) and soybean meal

Amino acid (wt. % protein)	BSFL-fed with OW1	BSFL-fed with OW2	Soybean meal
Aspartic acid	10.7	10.2	11.6
Threonine	4.6	4.6	4.0
Serine	4.9	5.1	5.3
Glutamic acid	12.2	12.7	18.4
Proline	6.3	6.7	5.3
Glycine	6.6	6.2	4.4
Alanine	7.2	8.2	4.6
Valine	6.0	5.9	4.4
Isoleucine	4.6	4.5	4.1
Leucine	8.0	7.7	7.6
Tyrosine	6.3	6.0	3.1
Phenylalanine	4.8	4.4	5.0
Histidine	3.2	3.0	2.7
Lysine	6.1	6.3	6.5
Arginine	5.1	5.2	8.1
Cysteine	0.4	0.7	2.1
Methionine	1.8	1.6	1.5
Tryptophan	1.2	0.9	1.2

proteins from microalgae are lower than those from insects, such as mealworm larvae and BSFL^{50,51}. BSFL exhibited higher productivity than the other feedstocks. Note that altering cultivation conditions and substrates could change the productivity of lipids and proteins from BSFL cultivation⁵².

The quality of proteins, particularly the composition of amino acids, is also an important factor in determining their suitability for livestock feed⁵³. For instance, the content and ratio of branched-chain amino acids such as leucine, valine, and isoleucine affect the growth performance and meat productivity of poultry⁵⁴. For pig farming, lysine is an important amino acid for growth and feed efficiency, while other amino acids, including methionine, cysteine, etc. are also important⁵⁵. The amino acid compositions of OW1/OW2-fed BSFL are shown in Table 2. As a reference, the amino acid composition of soybean meals was also analyzed. Note that soybean meals have been widely used for livestock feed⁵⁶. The amino acid compositions of BSFL-fed with OW1 and OW2 showed no significant differences. Moreover, their amino acid profiles were similar to soybean

meal, particularly in terms of branched-chain amino acids (leucine, valine, and isoleucine) and lysine, which are essential for broiler and pig farming. These results suggest that BSFL-derived proteins have high potential as an alternative to conventional livestock feed proteins, providing a comparable amino acid profile suitable for livestock farming.

Safety concerns are another issue for using BSFL-derived proteins as livestock feed. The European Union (EU) strictly regulates the rearing conditions for insects to ensure their biomass meets safety standards for use as livestock feed⁵⁷. Particularly regarding substrates for insect rearing, the EU regulations only permit the use of safe feed (such as commercial animal feed) for insects intended for livestock feeding. Given that OW1 and OW2 were collected from the pet feed industries, it is plausible that OW1 and OW2-fed BSFL could be used for livestock feeding. The heavy metal contents in OW1 and OW2-fed BSFL were also determined to ensure safety compliance for livestock feed applications. The regulated heavy metals (i.e., cadmium, lead, mercury, and arsenic) were not detected in BSFL-fed with OW1/OW2, suggesting that they meet regulatory safety standards for livestock feed applications.

In addition, given that black soldier flies (adult forms) do not have mouths, they have low possibility to transfer pathogens to humans or livestock⁵⁸. Note that the main route for pathogen transmission via insects is: (i) contact with contaminated waste (substrates), allowing pathogens to adhere to their bodies, (ii) movement to areas where humans and livestock reside, and (iii) transmission through blood-sucking and/or excrement⁵⁹. Since black soldier flies do not show feeding or blood-sucking behavior, their unique characteristic (the absence of functional mouthparts in the adult stage) prevents pathogen transmission through feeding. This natural biological trait enhances their safety as a feed source, reducing the risk of pathogen spread compared to other insect species.

The dual benefits of high productivity and the use of organic waste as feed for BSFL cultivation have economic and environmental value. Indeed, 1 ton of organic waste (expired pet feed) was converted into 95–132 kg of lipids and 87–111 kg of protein. The temperature for BSFL cultivation was set at 25 °C, although this rearing temperature was not the optimum temperature (31–36 °C) for BSFL growth⁶⁰. However, it is worth noting that 23–25 °C is in the range of suitable temperatures for raising meat produced from livestock, such as pig (21–24 °C)⁶¹, broiler (20–32 °C)⁶², and beef cattle (4–26 °C)⁶³. The shared temperature ranges of BSFL cultivation and livestock raising are advantageous because additional facilities for BSFL cultivation are not required; BSFL can be cultivated in the same facilities as current livestock farming systems. This enhances the economic viability of BSFL-based biorefineries. The concept of a BSFL-based biorefinery, which converts organic waste into livestock feed and biodiesel, has a high potential for contributing to economically viable, sustainable meat production.

Methods

BSFL cultivation

Two thousand BSFL (five days old) were cultivated in a plastic box (width 45, length 31, and height 18 cm). One hundred grams of feed (33 g organic waste (either OW1 or OW2), 67 g water) was provided in each box at two-day intervals. The cultivation temperature was set at 23–25 °C, and the humidity was maintained at 60–70%. The plastic boxes were covered with a blanket to maintain temperature and prevent light exposure. Twenty BSFL were randomly harvested at four-day intervals to determine the average fresh weight of the larvae. To determine the average dry weight of the larvae, the harvested BSFL were dried at 95 °C in the oven. The dried BSFL was ground and used for biomass analysis.

Organic waste and BSFL biomass analyses

To determine crude protein contents in organic waste and BSFL, the nitrogen-to-protein conversion factor of 6.25 for organic waste and 4.76 for BSFL²⁷. The nitrogen content was determined using an elemental analyzer (EA3100, EUROVECTOR, Italy). 0.50 ± 0.05 mg of sample was loaded into the equipment, and sulfanilamide was used as the standard for the measurement.

Lipids in the organic waste were extracted using a Soxhlet extractor with *n*-hexane as the solvent. A thimble filter was filled with 100 g of specimen and extracted for 24 h at 80 °C. To confirm the FA composition in BSFL, a direct conversion method was used to convert FA into fatty acid methyl esters (FAMES)^{25,26}. In detail, 10 mg of sample, 320 mg of silica (pore size 60 Å), and 200 µL of methanol were loaded in a bulkhead. The sample-loaded bulkhead was placed in a tube furnace, increasing the temperature to 380 °C. A K-type thermocouple (OMEGA, USA) monitored the bulkhead internal temperature. After cooling the bulkhead, the FAMES were recovered using dichloromethane. A gas chromatography/flame ionization detector (GC/FID 8890, Agilent, Santa Clara, CA, USA) was used to analyze the FAMES. A DB-WAX column (ID: 0.25 mm, L: 30 m, and film thickness: 0.25 µm) was employed in the GC/FID. The Supelco 37 Component FAME Mix served as the standard solution for FAME analysis.

Thermogravimetric analysis (TGA) was performed using a Netzsch Jupiter F5 TGA. The TGA test was conducted under N₂ and CO₂ with a temperature gradient of 10 °C min⁻¹; the analyzed temperature range was 50–800 °C, with the analysis temperature adjusted using the built-in software. During the analysis, the flow rates of N₂ and CO₂ were maintained at 100 mL min⁻¹. The sample loading amount for TGA tests was 10 mg ± 0.01 mg.

The analysis of amino acids was conducted using ion exchange chromatography with a ninhydrin post-column reaction. For the 16 components of amino acids, 0.2 g of the sample was placed in a decomposition tube, followed by the addition of 10 mL of 6 N HCl. The mixture was then subjected to nitrogen gas injection and underwent hydrolysis at 110 °C for 24 h. The resulting hydrolysate was concentrated using a vacuum concentrator, adjusted to 50 mL with 0.2 M sodium citrate buffer, and filtered with a 0.2 µm cellulose acetate syringe filter. For sulfur-containing amino acids, methionine, and cysteine, performic acid oxidation was employed, while tryptophan was analyzed with an alkaline hydrolysis method.

Data availability

Data will be made available on request.

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Competing interests

The authors declare no competing interests.

Additional information

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