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Impact of substrate type and growth stage on nutrient composition and convergence efficiency of *Hermetia illucens* larvae

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Abstract
This study investigates the bioconversion efficiency and nutrient accumulation in black soldier fly (BSF; *Hermetia illucens*), focusing on the effects of feeding on two different substrates (tofu by-products and food waste) and harvesting at two developmental stages (larvae and prepupae) within a 2 × 2 factorial arrangement. The growth performance, conversion efficiency, nutrient composition, amino acid profile, fatty acid composition, and nutrient composition of BSF meal were assessed. Results indicated that BSF reared on tofu by-products exhibited superior weight gain compared to those fed food waste, with significant enhancements observed in weight, length, and width upon harvesting at the prepupae stage. Moreover, tofu by-products promoted higher bioconversion rates, protein conversion efficiency, and lipid yield, while food waste favored lipid conversion. Analysis of nutrient composition revealed higher crude protein content in BSFs fed tofu by-products, with elevated levels of crude protein, ether extract, and chitin in prepupae-stage BSFs. Higher concentrations of isoleucine, leucine, and tryptophan were observed in tofu by-product-fed BSF. Conversely, BSFs harvested at the prepupae stage exhibited increased levels of threonine, alanine, and tyrosine, regardless of substrate. Higher proportions of α-linolenic acid (C18:3n3) and docosahexaenoic acid (C22:6n3) were observed in tofu by-product-fed BSF. Conversely, BSF harvested at the larval stage displayed higher levels of saturated fatty acids, including lauric acid (C12:0) and myristic acid (C14:0). In conclusion, tofu by-products emerged as a promising substrate for enhancing essential amino acid and unsaturated fatty acid content in BSF, while harvesting at the prepupae stage offered advantages in nutrient density and storage stability of the harvested biomass.
Keywords: Food waste, Tofu, Bioconversion, Larvae, Prepupae, Black soldier fly

INTRODUCTION

Sustainable agriculture strives to meet the world nutritional needs while minimizing environmental degradation and resource depletion [1,2]. Insects have emerged as promising candidates owing to their

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Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions
Conceptualization: Ha SH, Hosseindoust A, Mun JY.
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Ethics approval and consent to participate
The animal care and experimental protocols used in this study received approval by the Institutional Animal Care and Use Committee of Kangwon National University (Ethical code: 210503-6).

environmental sustainability and efficiency [3]. Among these alternatives, the black soldier fly (BSF; *Hermetia illucens*) has garnered considerable attention in recent years due to its potential to address sustainability challenges in agriculture [4,5]. The larvae of BSF offer numerous advantages as a protein source for animal nutrition, offering a viable solution to food security concerns. A primary advantage lies in BSF remarkable efficiency in converting organic waste into protein biomass [4,6,7]. These voracious larvae possess the ability to decompose a wide array of organic substrates such as food waste, agricultural residue, and animal manure into nutrient-rich biomass [2,3,8], presenting a sustainable solution to waste management while augmenting feed production. The larvae emit fewer greenhouse gases, rendering BSF cultivation a more eco-friendly alternative to traditional livestock farming practices [9].

Economically, BSF production offers cost-effective advantages over conventional livestock rearing [7]. Notably, by-products from tofu processing, such as tofu whey, represent an underutilized feed resource for BSF rearing [3]. The abundance of tofu waste, estimated at millions of tons annually in select regions, underscores the potential for BSF cultivation to mitigate waste disposal challenges while generating valuable protein biomass [3].

Nevertheless, the nutritional composition and growth performance of BSF larvae are influenced by their diet, particularly botanical waste substrates [10–12]. Optimization of dietary inputs is pivotal to maximizing protein production efficiency per unit of organic waste, thereby enhancing the sustainability and cost-effectiveness of BSF-based feed production [8]. Moreover, the nutrient composition of BSF meal can be under the influence of the growth stage harvest including larvae and prepupae [13]. Thus, a systematic evaluation of BSF's growth dynamics, conversion efficiency, and larval quality across different botanical waste substrates is imperative to identify optimal feeding regimes.

This study endeavors to assess the growth performance, conversion efficiency, and larval quality of BSF reared on various botanical waste substrates, namely food waste and tofu by-products. By elucidating the effects of substrate type and developmental stage (larvae or prepupae) on nutrient composition, weight, length, development time, substrate consumption index, conversion efficiency, and fatty acid composition, this research aims to contribute to the optimization of BSF rearing practices for sustainable and efficient protein production in the context of waste valorization and animal feed supplementation.

MATERIAL AND METHODS

Larvae rearing control

The experiment was conducted at a commercial BSF farm located in Eui-sung, Gyeongsangbuk-do, Korea. Prior to hatching, the BSF insects used in this study were sourced from the farm. The trial colony was meticulously maintained under controlled environmental conditions, with a temperature set at 27 ± 0.3℃, relative humidity maintained at 65 ± 8%, and a photophase of 16 hours of natural light supplemented with LED lighting providing an intensity of 6,000 lux. For the experimental setup, a total of 30 egg clutches were utilized, each containing approximately 50,000 larvae. The average weight of the egg clutches was measured at 24.16 ± 0.91 mg per clutch, with an average egg weight of 0.027 mg. The egg clutches were further divided into four treatment groups, comprising two different substrates (tofu by-product and food waste; Table 1) and two distinct growth stages (larvae and prepupae). Each treatment group consisted of 10 replicates, housed within experimental boxes measuring 1,064 × 507 × 320 mm (length × width × height). The trial continued until the end of the prepupal stage, just prior to the pupal stage transition. Egg counting within each clutch was performed according to the methodology outlined by Georgescu et al. c, employing an Alpha model

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Table 1. Nutrient composition of botanical waste used in trial

Substrate (%)	Food waste	Tofu By-product
Dry matter	16.95	23.96
Crude protein	19.68	30.52
Ether extract	21.65	12.95
Ash	6.85	7.96

binocular magnifier with 7× to 45× magnification. To ensure accuracy, the eggs were dispersed in 70% ethanol and subsequently captured and enumerated through photographic means utilizing the ClickMaster2000 1.0 software, accessible at https://www.thregr.org/~wavexx/. This meticulous process facilitated precise egg counting, essential for subsequent experimental procedures and data analysis.

Assessment of larval growth performance

To assess the growth performance of BSF larvae, measurements were taken at the time of harvest, including weight, length, width, and survivability. In order to standardize the comparison across harvest days, 100 larvae were randomly selected for measurement beginning on day 10, and continuing until the average larval weight reached 190 mg. The larval yield, indicative of survivability on the harvest day, was calculated using the following equation [3]:

$$\text{Larval yield } \% = \frac{\text{Larvae}_F}{\text{Larvae}_I} \times 100 \qquad (1)$$

where:

Larvae_F represents the number of larvae at the end of the trial. Larvae_I represents the number of larvae at the initiation of the trial.

This approach allowed for a comprehensive evaluation of larval growth and survival dynamics, essential for assessing the efficacy of different experimental treatments. Additionally, the utilization of standardized measurement protocols ensured accuracy and reliability in the assessment of larval performance across varying experimental conditions.

Substrates preparation

The substrates utilized in this study were procured from local farms and facilities to ensure relevance to regional waste streams. Prior to their use, dried substrates were moistened with water to achieve a target moisture content of 65%. Egg blocks were then positioned on two distinct substrates: food waste and tofu by-products. The food waste substrate consisted of organic waste collected from households, kitchens, and local dining establishments through a municipal waste collection facility located in Eui-sung, Gyeong-sangbuk-do, Republic of Korea. Tofu by-products, comprising soybean curd residue, were sourced from a local tofu manufacturing facility situated in Kang-reung, Kangwon-do, Republic of Korea. To prevent fermentation and ensure uniformity across substrates, the materials were promptly dried using an industrial drying apparatus (KAPD-A098D, CNT), operating at a temperature of 60℃ for 48 hours, until the moisture content was reduced to less than 8%. Upon commencement of the experiment, water was applied to the substrates to reach the desired moisture level of 65%. Each experimental box was provisioned with a total of 20 kg (dry matter basis) of substrate. To create a suitable environment for larval development and prevent drowning, a layer of 1 kg of recycled paper pulp and 600 g of sawdust was spread atop the substrates. This bedding layer facilitated larval movement and provided an additional substrate for microbial activity, which may contribute to the decomposition process.

Chemical composition analysis

The chemical composition of the harvested larvae from each treatment was determined to assess their nutritional profile. The contents of dry matter (DM), crude protein (CP), ether extract (EE), crude ash (Ash), and chitin were analyzed using standardized methods outlined by the Association of Official Analytical Chemists [14]. Initially, fresh larvae were weighed to establish baseline measurements. Subsequently, the larvae were subjected to drying in an oven set at 85℃ for a duration of three days to determine the DM content, following the procedure outlined in method 930.15 of the AOAC [14]. To quantify the protein content, AOAC official method 990.03 was employed, utilizing the Kjeldahl method. This method entails the digestion of the sample with sulfuric acid to release nitrogen, followed by conversion of the nitrogen to ammonia. The ammonia is then distilled and titrated to determine the nitrogen content, which is subsequently utilized to calculate the protein content [14]. Similarly, the ether extract content was determined using AOAC official method 990.15, involving the extraction of lipid content from the sample with a suitable solvent, followed by evaporation of the solvent to obtain the lipid content [14]. The gross energy of BSF larvae was measured using a bomb calorimeter (Model 1261, Parr Instrument), providing insight into the calorific value of the larvae. Following analysis, any remaining samples were promptly stored at −20℃ for future analysis and reference, ensuring preservation of sample integrity for subsequent investigations. The amino acid (AA) composition of BSF meal was assessed using high-performance liquid chromatography (HPLC) with a Waters 486 system (Waters). This analysis was conducted following acid hydrolysis, as described by Lee et al. [15], to release the amino acids from the protein matrix for quantification. To ensure comprehensive analysis, the determination of methionine and cysteine was performed after oxidation with performic acid, following the methodology outlined by Kim et al. [16].

Larval convergence efficiency evaluation

To assess the convergence efficiency of larvae reared on different botanical wastes, several parameters including bioconversion rate (BCR), waste reduction rate (WR), protein conversion rate (PCR), lipid conversion rate (LCR), protein yield (PY), lipid yield (LY), and mass distribution patterns were evaluated using the following equations.

The BCR was calculated as:

$$\mathrm{BCR\%} = \frac{\mathrm{DM_F} - \mathrm{DM_I}}{\mathrm{DM_S}} \times 100 \tag{2}$$

where $\mathrm{DM_F}$ represents the dry matter of BSF larvae at harvest day, $\mathrm{DM_I}$ represents the dry matter of BSF larvae at the initiation of the trial, and DMS represents the total dry matter of the substrate.

The WR was determined by:

$$\mathrm{WR\%} = \frac{\mathrm{DM_S} - \mathrm{DM_R}}{\mathrm{DM_S}} \times 100 \tag{3}$$

where $\mathrm{DM_S}$ represents the total dry matter of the substrate and $\mathrm{DM_R}$ represents the residual dry matter of the substrate after larval consumption.

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The PCR was calculated as:

$$PCR\ \% = \frac{DM_F \times Protein_L\ \%}{DM_S} \times 100 \tag{4}$$

where $protein_L$ % represents the protein content in the larvae, DM_F represents the dry matter of BSF larvae at harvest day, and DMS represents the total dry matter of the substrate.

The LCR was determined by:

$$LCR\% = \frac{DM_F \times EE_L\ \%}{DM_S} \times 100 \tag{5}$$

where EE_L % represents the lipid content in the larvae, DM_F represents the dry matter of BSF larvae at harvest day, and DM_S represents the total dry matter of the substrate.

The PY was calculated as:

$$PYkg / c = g\ of\ DM_{F\ yield} \times CP_L\ \% \tag{6}$$

where DM_F yield represents the dry matter of BSF larvae at harvest, and CP_L % represents the crude protein content in the larvae.

The LY was determined as:

$$LYkg / c = g\ of\ DM_{F\ yield} \times EE_L\ \% \tag{7}$$

where DM_F yield represents the dry matter of BSF larvae at harvest, EE_L % represents the ether extract content in the larvae, and 'c' represents the clutch size (50,000 larvae).

Fatty acid composition

The determination of fatty acid content was conducted following the methodology outlined by Georgescu et al. [17]. For analysis, 10 g of meal sample was collected and subsequently placed on a clean substrate for a 24-hour period. Following this, the samples were washed with 70% ethanol to remove any impurities and then stored at −80℃ to maintain sample integrity. Extraction and identification of fatty acid methyl esters (FAME) from BSF larvae fats were performed using gas chromatography with mass spectrometry detection, adhering to established standards including AOAC-969.33 [14], ISO 3657: 2002, ISO 12966-2: 2011, and ISO 12966-2: 2017. The analytical process involved saponification of the fat, achieved by subjecting the samples to methanolic sodium hydroxide solution (0.5 M) at 210℃ on a sand bath with a reflux rate of 1 drop/s. Subsequently, esterification with a 15% vol boron trifluoride catalyst and cooling with hexane were carried out. Gas chromatographic analysis was conducted using a Perkin Elmer chromatographic system equipped with a mass spectrometer detector (GC-MS). This system featured an Elite-Wax chromatographic column with a stationary polar phase polyethylene glycol measuring 30 m in length, 0.25 mm in internal diameter, and a film thickness of 1.0 μm. Operating parameters included an injection port temperature of 220℃, an injected sample volume of 1.0 μL, a helium carrier gas flow rate of 1.5 mL/min, and a splitting ratio of 40:1. The mass spectrometer settings comprised a transfer line temperature of 150℃, a source temperature of 150℃, a multiplier of 1,500, and a solvent delay of 0–1.5 min. Quantification of fatty acid concentration was achieved by comparing the relative retention time of FAME to the certified standard Mix FAME Supelco. The individual fatty acid concentrations were expressed as a percentage of the total identified FAME. Moreover, key ratios between various fatty acid categories, including total saturated fatty acids (SFA) and total unsaturated fatty acids, were calculated. Additionally, the ratios between fatty acids from the n-6 and n-3 series were determined to further elucidate the nutritional profile of the BSF biomass.

Statistical analysis

The data were subjected to analysis employing a 2 × 2 factorial arrangement utilizing the General Linear Model procedure of SAS (version 9.4, 1996, SAS Institute). Differences in various parameters including growing performance, conversion efficiency, nutrient composition, amino acid, and fatty acid concentrations among the treatments were assessed using a two-way ANOVA test. To further elucidate the differences between treatments, the Tukey test was employed to compare the means of the treatments. Statistical significance was determined at a threshold of $p < 0.05$. Moreover, a p-value less than 0.1 was considered indicative of a trend. The results are expressed as the mean ± SD, providing insights into the central tendency and variability of the data.

RESULTS

Growth performance

The BSF larvae reared on tofu by-products exhibited significantly higher ($p < 0.05$) weight gain compared to those fed with food waste (Table 2). Moreover, harvesting at the prepupae stage resulted in notable enhancements in weight, length, and width of BSF, indicating superior growth performance compared to larvae-harvested BSF, which displayed higher survival rates. The BSF larvae reared on tofu by-products exhibited significantly higher ($p < 0.01$) BCR, PCR, PY, and LY compared to those fed with food waste (Table 2), however, LCR was lower in BSF fed tofu by-products compared with food waste. Moreover, harvesting at the prepupae stage resulted in lower BCR compared to larvae harvested BSF, however, harvesting at the prepupae stage showed higher PY, LCR, PY, and LY compared to larvae harvested BSF.

Table 2. Effects of substrate (SUB) and development stage (STG) on growth performance of black soldier fly

Treatments	Food waste		Tofu by-product		SEM	*p*-value		
	Larvae	Prepupae	Larvae	Prepupae		SUB	STG	SUB×STG
Larvae growth								
Weight, mg	177.92	191.05	194.97	209.39	2.178	< 0.001	< 0.001	0.802
Length, mm	13.83	15.20	14.20	15.21	0.132	0.305	< 0.001	0.342
Width, mm	2.79	2.94	2.84	2.95	0.021	0.477	0.002	0.614
Survival rate, %	80.14	77.53	77.97	75.50	0.575	0.056	0.022	0.948
Substrate consumption, %	69.01	77.65	72.16	79.55	0.971	0.090	< 0.001	0.671
Conversion efficiency rate, %								
Bioconversion	12.10	11.61	12.67	12.36	0.084	< 0.001	0.001	0.440
Protein conversion	6.12	6.93	9.91	11.02	0.040	< 0.001	< 0.001	0.069
Lipid conversion	5.88	6.64	4.45	4.96	0.025	< 0.001	< 0.001	0.082
Protein yield, kg/c	1.13	1.32	1.32	1.47	0.029	0.008	0.005	0.859
Lipid yield, kg/c	1.03	1.16	1.13	1.26	0.022	0.039	0.006	0.902

Each value is the average of 10 replicates.

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Nutrient composition of meal

The analysis revealed no significant differences in dry matter, gross energy, or ash content across different substrates and developmental stages (Table 3). However, BSFs fed on tofu by-products exhibited a higher crude protein content compared to those fed with food waste. Furthermore, harvesting at the prepupae stage led to elevated levels of crude protein, ether extract, and chitin, indicating enhanced nutrient composition in prepupae-stage BSF.

Amino acid profile of the meal

Amino acid profiling demonstrated distinct compositions in BSF fed with different substrates and harvested at different developmental stages (Table 4). BSFs reared on tofu by-products showed increased concentrations of isoleucine, leucine, tryptophan, alanine, and proline. In contrast, BSFs harvested at the prepupae stage displayed higher levels of threonine, tryptophan, alanine, serine, and tyrosine, indicating variations in amino acid profiles influenced by both substrate type and developmental stage.

Fatty acid composition of meal

Fatty acid analysis revealed variations in the composition of BSF lipids based on substrate and developmental stage (Table 5). BSFs fed on tofu by-products exhibited higher ratios of C10:0, C12:0, C14:0, C14:1, C18:3n3, and C20:1 fatty acids, while those consuming food waste showed a higher content of C18:4. Additionally, SFA such as C12:0, C15:0, C17:0, and C18:1 were more abundant in BSFs harvested at the prepupae stage.

DISCUSSION

The higher weight gain observed in BSF larvae reared on tofu by-products compared to those fed with food waste suggests that the nutrient composition and bioavailability in tofu by-products are more conducive to larval growth. The higher protein in tofu by-products may facilitate improved growth rates. The enhancements in weight, length, and width of BSF when harvested at the prepupae stage could be attributed to the larval maturation process. As larvae transition into prepupae, they are in a state of maximal nutrient accumulation and tissue development [7]. This stage is also characterized by efficient energy storage and nutrient conversion in preparation for metamorphosis [13], which may explain the observed improvements in growth performance at this developmental stage. In contrast, the higher survival rates in larvae harvested BSF as opposed to those harvested at the prepupae stage may be linked to the life cycle and physiological state of the

Table 3. Effects of substrate (SUB) and development stage (STG) on nutrient composition of black soldier fly

Treatments	Food waste		Tofu by-product		SEM	*p*-value		
	Larvae	Prepupae	Larvae	Prepupae		SUB	STG	SUB × STG
DM, %	40.60	42.73	41.57	42.97	0.689	0.662	0.207	0.792
GE, kcal	4,315	4,343	4,388	4,410	30.0	0.253	0.684	0.964
CP, % of DM	39.12	41.66	41.63	43.41	0.326	0.002	0.002	0.569
EE, % of DM	35.53	36.67	35.76	37.02	0.283	0.617	0.041	0.920
Ash, % of DM	7.04	7.24	7.10	7.29	0.167	0.872	0.564	0.916
Chitin, % of DM	7.85	8.45	8.15	8.60	0.125	0.257	0.049	0.882

Each value is the average of 10 replicates.

DM, dry matter; GE, gross energy; EE, ether extract.

Table 4. Effects of substrate (SUB) and development stage (STG) on amino acid profile of black soldier fly

Treatments	Food waste		Tofu by-product		SEM	*p*-value		
	Larvae	Prepupae	Larvae	Prepupae		SUB	STG	SUB×STG
Essential amino acid, %								
Arginine	1.86	1.95	1.74	1.93	0.036	0.361	0.061	0.520
Histidine	1.06	1.07	1.19	1.11	0.028	0.296	0.062	0.710
Isoleucine	1.45	1.16	1.66	1.83	0.031	0.002	0.016	0.958
Leucine	2.39	2.44	2.53	2.68	0.030	0.005	0.114	0.426
Lysine	2.81	2.92	2.84	2.93	0.062	0.836	0.423	0.931
Methionine	0.59	0.64	0.54	0.58	0.020	0.219	0.321	0.951
Phenylalanine	1.07	1.21	1.14	1.23	0.033	0.109	0.164	0.459
Threonine	1.36	1.48	1.38	1.58	0.022	0.205	0.002	0.400
Tryptophan	1.97	2.21	2.23	2.32	0.030	0.007	0.015	0.243
Valine	2.53	2.58	2.59	2.74	0.076	0.479	0.513	0.716
Non-essential amino acid, %								
Alanine	2.46	2.56	2.63	2.77	0.029	0.004	0.049	0.680
Aspartate	3.16	3.49	3.34	3.46	0.056	0.522	0.054	0.355
Cysteine	0.26	0.28	0.31	0.32	0.018	0.177	0.747	0.927
Glutamate	3.92	4.02	3.82	4.01	0.175	0.863	0.684	0.893
Glycine	1.93	2.06	2.11	2.13	0.031	0.062	0.243	0.335
Proline	2.50	2.64	2.72	2.73	0.026	0.006	0.183	0.203
Serine	1.37	1.58	1.42	1.55	0.025	0.894	0.002	0.449
Tyrosine	1.97	2.23	2.14	2.25	0.024	0.070	0.001	0.117

Each value is the average of 10 replicates.

larvae. Larval stages possess more resilience and adaptability to varying environmental conditions and nutrient availability compared to prepupae [18]. This higher adaptability results in increased survival rates when harvesting at the larval stage [8]. Overall, these results highlight the importance of substrate choice and timing of harvest in optimizing BSF growth performance. The mode of action related to nutrient availability, larval development, and life cycle dynamics are central to understanding the differences observed in growth performance.

The lack of significant differences in dry matter, gross energy, and ash content across different substrates and developmental stages suggests that the overall energy density and mineral content of BSF biomass remain relatively consistent regardless of dietary inputs or harvest timing. However, BSF fed tofu by-products demonstrated higher crude protein content compared to those fed food waste. This difference can be attributed to the higher protein levels in tofu by-products [3]. Harvesting BSF at the prepupae stage led to increased levels of crude protein, ether extract, and chitin. This suggests that as BSF larvae mature and transition to the prepupae stage, they undergo biochemical and physiological changes that enhance their nutrient composition [19]. The increase in crude protein and ether extract may be due to the prepupae preparation for metamorphosis, which involves accumulating energy and nutrient reserves. Additionally, elevated chitin levels are associated with the development of the exoskeleton as the insects approach the pupal stage [20].

The amino acid profile of BSF is a crucial aspect of its nutritional value and potential applications as animal feed [3,13]. BSF reared on tofu by-products exhibited increased concentrations of isoleucine, leucine, tryptophan, alanine, and proline compared to those fed with food waste. This suggests that tofu by-products provide a rich source of essential and conditionally essential amino acids, contributing to a more balanced and nutritious profile for the larvae. The

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Table 5. Effects of substrate (SUB) and development stage (STG) on fatty acid composition of black soldier fly

Treatments	Food waste		Tofu by-product		SEM	*p*-value		
	Larvae	Prepupae	Larvae	Prepupae		SUB	STG	SUB × STG
Fatty acid, %								
C10:0	0.88	0.91	0.94	0.97	0.009	0.006	0.160	0.827
C12:0	22.22	23.28	23.72	24.90	0.174	< 0.001	0.004	0.871
C14:0	4.38	4.63	4.71	4.68	0.042	0.035	0.238	0.111
C14:1	0.12	0.10	0.16	0.15	0.010	0.032	0.437	0.814
C15:0	0.14	0.16	0.16	0.20	0.008	0.051	0.033	0.454
C16:0	15.43	16.36	14.64	14.35	0.335	0.050	0.641	0.373
C16:1	3.25	3.31	3.29	3.25	0.017	0.882	0.730	0.125
C:17:0	0.22	0.26	0.19	0.23	0.007	0.065	0.015	0.861
C18:0	1.90	2.27	2.03	2.14	0.045	0.985	0.016	0.160
C18:1	24.10	23.41	23.89	23.06	0.268	0.607	0.174	0.893
C18:2n6	19.25	17.04	17.86	17.51	0.328	0.496	0.065	0.170
C18:3n3	2.16	2.11	2.23	2.25	0.022	0.036	0.811	0.392
C18:4	0.90	0.91	0.70	0.74	0.007	< 0.001	0.105	0.527
C20:1	0.24	0.27	0.45	0.47	0.010	< 0.001	0.345	0.811
C20:4n6	0.26	0.21	0.22	0.21	0.009	0.290	0.067	0.252
C20:5n3	1.36	1.28	1.23	1.31	0.017	0.121	0.980	0.028
Other	2.01	2.14	2.35	2.30	0.049	0.016	0.685	0.374
SFA	45.17	47.86	46.39	47.46	0.400	0.612	0.030	0.325
USFA	53.64	50.77	52.38	51.25	0.417	0.647	0.026	0.311

Each value is the average of 10 replicates.
SFA, saturated fatty acids; USFA, unsaturated fatty acid.

higher levels of these amino acids may result from the nutrient-rich nature of tofu by-products, which could provide an optimal blend of proteins and other macronutrients for larval development. In contrast, BSF harvested at the prepupae stage displayed elevated levels of threonine, tryptophan, alanine, serine, and tyrosine compared to those harvested at the larval stage. This shift in amino acid composition during the prepupae stage is linked to the physiological changes the insects undergo in preparation for metamorphosis [7,13,21]. The variation in amino acid profiles based on substrate type and developmental stage underscores the importance of optimizing these factors for specific applications of BSF biomass.

The fatty acid composition of BSF is a critical aspect of its nutritional quality and potential applications in various industries, including animal feed and biodiesel production [2,13]. BSF fed on tofu by-products exhibited higher ratios of specific fatty acids, including C10:0, C12:0, C14:0, C14:1, C18:3n3, and C20:1, compared to those consuming food waste. These differences in fatty acid composition may be due to the lipid profiles present in the respective substrates. The observed higher content of C18:4 in BSF consuming food waste suggests preferential utilization or accumulation of this fatty acid in response to the substrate lipid content. Additionally, SFA such as C12:0, C15:0, C17:0, and C18:1 were more abundant in BSF harvested at the prepupae stage. This is attributed to metabolic changes associated with larval development and maturation into prepupae [19]. As the larvae transition to the prepupal stage, they undergo metabolic shifts to support pupation and subsequent adult emergence [2,19], leading to alterations in fatty acid synthesis and accumulation. The variations in the fatty acid composition of BSF biomass have significant implications for its nutritional value and suitability for various applications [22]. For instance, certain fatty acids, such as omega-3 and omega-6 fatty acids, are essential for animal health [1,2,11] and contribute to the nutritional quality of BSF-based feed formulations.

BSF meal derived from larvae reared on tofu by-products exhibited higher gross energy compared to meal from food waste-fed BSF. This difference could be attributed to the higher energy density of the tofu by-product substrate, which may have contributed to enhanced energy accumulation in the larvae. As a result, the meal from tofu by-product-fed BSF could provide a more energy-rich feed ingredient. Moreover, meals from prepupae-stage BSF demonstrated enhanced gross energy, crude protein, and chitin content. These changes can be due to the physiological and biochemical transformations that occur as the larvae mature into prepupae [2,6,19]. The increased gross energy may result from the accumulation of energy reserves to support metamorphosis, while the higher crude protein and chitin levels are associated with tissue development and exoskeleton formation during the pupal transition [2,20]. The distinct fatty acid profile favoring C12:0, C18:3n3, and C22:6n3 in meals from prepupae-stage BSFs suggests alterations in fatty acid metabolism as the larvae approach the pupal stage. These specific fatty acids may be selectively synthesized or accumulated in response to developmental cues, contributing to the observed fatty acid composition. The higher levels of SFA in prepupae-stage BSF meal could reflect shifts in metabolic pathways aimed at supporting pupation and adult emergence [9,10]. Saturated fatty acids play a role in providing stable energy sources during this critical developmental phase [2,10,17]. Understanding the mode of action underlying these variations in nutrient composition can help optimize BSF meal production for targeted applications. The higher gross energy and crude protein content in prepupae-stage BSF meal increases its value as a feed ingredient for high-performance livestock.

CONCLUSION

Our results demonstrate that substrate type significantly influences the growth performance, nutrient composition, and fatty acid profile of BSF larvae and prepupae. Larvae reared on tofu by-products exhibited superior growth performance and higher levels of crude protein, while those fed food waste displayed distinct fatty acid compositions. Additionally, harvesting BSF at the prepupae stage resulted in enhanced nutrient composition and fatty acid profiles, indicating the importance of the developmental stage in optimizing biomass quality. Furthermore, it was revealed that by understanding the mode of action underlying these variations, we can develop strategies to optimize BSF production systems for farm animal nutrition, sustainable agriculture, and waste management.

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