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Macronutrient-specific organic waste bioconversion by black soldier fly (*Hermetia illucens*) larvae: Degradation efficiency, larval development, and chitin-related implications for catfish (*Clarias batrachus*) growth

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ABSTRACT

Organic waste management in institutional settings presents a persistent challenge due to the fluctuating macronutrient composition of daily waste streams. This study investigated the effect of macronutrient composition on black soldier fly larvae (BSFL, *Hermetia illucens*) bioconversion performance and larval development using four substrate types: tofu waste (protein-rich), leftover rice (carbohydrate-rich), green cabbage (fiber-rich), and a mixed substrate of equal proportions of all three. Conducted as part of an organic waste management initiative at Universitas Sumatera Utara, 7-day-old BSFL were cultivated over a 21-day feeding period, with larval length, weight, color, pre-pupal development, and chitin formation assessed at regular intervals. A preliminary antinutritional evaluation was further conducted using catfish (*Clarias batrachus*) fed a composite diet of 50% BSFL pupae and 50% commercial fish feed. Results demonstrated that substrate macronutrient composition significantly influenced all measured parameters ($p < 0.05$). Tofu waste exhibited the highest bioconversion rate, with complete substrate consumption within approximately 10 hours per feeding cycle, while green cabbage showed persistent residual substrate throughout cultivation. The mixed substrate achieved the highest total larval length at harvest (17.298 ± 3.341 mm), while tofu-fed larvae recorded the highest body weight. Pre-pupal formation was most advanced in the tofu and mixed-substrate treatments, whereas green cabbage larvae failed to initiate pre-pupal transformation within the cultivation window. Chitin yield was highest in mixed (18.136 ± 0.941%) and tofu (17.219 ± 0.530%) treatments at Day 28, while FTIR analysis confirmed that all substrates produced structurally identical α-chitin, which confirms substrate waste composition influences chitin quantity but not quality. A preliminary antinutritional trial indicated that partial substitution with chitin-bearing BSFL pupae (estimated 8–9% dietary chitin) significantly suppressed catfish growth over 14 days; as this short-term trial could not isolate the effect of chitin from other dietary differences, the finding is preliminary and requires confirmation through longer, dose-controlled studies.

Keywords: BSF larvae, chitin yield, substrate variation, catfish growth, organic waste.

INTRODUCTION

The increasing generation of organic, inorganic, and municipal waste continues to challenge existing waste management systems, particularly in developing regions, like Indonesia, where infrastructure and collection service efficiency remain limited. Recent studies have also highlighted the need for improved waste management strategies and resource-recovery approaches in both rural and urban environments (Bredun et al., 2024). To be more specific , Indonesia currently faces a mounting crisis due to the vast quantities of organic waste generated from households, traditional markets, as well as the food and beverage sectors (Low Carbon Development Indonesia, 2021). A substantial proportion of the organic waste remains inadequately managed and is ultimately disposed of in landfills, reducing landfill capacity and constraining their long-term availability for future waste disposal. The landfill burden is increasing due to rapid population growth, accelerating urbanization, evolving consumption patterns, and a lack of societal knowledge and responsibility, which have led to a massive increase in unutilized waste production, with organic materials, such as food scraps and yard waste, making up the largest share, often exceeding 50 % of total municipal solid waste reported in most of Indonesia waste facilities (Wikurendra, et al. 2024). The National Waste Management Information System of the Ministry of Environment and Forestry, the Republic of Indonesia, has reported that the total waste production in 2024, where the total amount of waste generated reached 33,820,687.54 tons, with the composition of organic waste reaching 39.26 % of the total generation (Kementrian Lingkungan Hidup Republik Indonesia, 2026). To be more specific, in our city, Medan, the capital of North Sumatra the total waste production can amount to hundreds of tons per day, straining existing waste management infrastructure and leading to widespread improper disposal (Hanafiah and Muhdi, 2022). The condition was worsened by the fact that the government-funded waste service could not cover all areas due to limited infrastructure, especially transportation, which made most of the waste uncollected and left in the residential area. The inadequate management of organic waste, frequently dumped in open landfills, burned, or left to decompose, poses serious threats to both the environment and public health. Unmanaged organic waste contributes to air, water, and soil pollution, releases greenhouse gases like methane, and increases the risk of flooding and vector-borne diseases (Yuan and Zoungrana, 2025).

Comprehensive waste management strategies and policies are essential to address the growing waste challenges in Indonesia. The government of the Republic of Indonesia should prioritize waste management, particularly in development of infrastructure as well as expansion of waste collection and service coverage. However, the implementation remains challenging due to Indonesia's archipelagic geography, which limits accessibility and complicates the transportation of waste from dispersed regions to centralized landfill sites. In the case of Sumatra Island, the extensive service area and significant distances between regions hinder the effectiveness of interconnected waste management systems, as infrastructural limitations constrain the integration of facilities. Therefore, decentralized integrated waste facilities have emerged as a more practical solution for handling locally generated waste, optimizing resource utilization while reducing the burden on government-led collection and transportation systems. Moreover, such facilities (integrated waste facilities) offer opportunities to enhance waste value by integrating multiple treatment technologies, which can convert non-valuable waste into a highly valuable or commercial one (Kurniawan et al., 2026). This idea has basically been implemented in authors' institution, Universitas Sumatera Utara (USU), where USU has developed an integrated waste management facility that processes locally generated waste within the campus. The coverage area includes the organic and inorganic waste, where most of the generated waste has been successfully utilized, with a small amount of waste ending up in the landfills.

To be more specific on organic waste, the waste facilities of Universitas Sumatera Utara have utilized black soldier fly larvae (*Hermetia illucens*) to facilitate the bioconversion of organic waste, transforming the organic waste into larval biomass and frass. The obtained bioconversion product could be further utilized as aquaculture feed (BSFL) and organic fertilizer (Frass). The use of BSFL in the organic waste bioconversion was based on the widely reported studies that reported the successful bioconversion of diverse organic waste streams and transformed the waste into valuable biomass rich in protein, lipids, and bioactive compounds, highlighting the strong potential for circular economy applications (Anokye, 2025). As mentioned, the biodegradation performance of BSFL in waste degradation has been widely reported across various waste streams, demonstrating their ability to convert organic waste into valuable biomass and frass, with nutrient profiles reflecting the original substrate composition (Grossule et al., 2025; Singh et al., 2021; and Tao et al., 2023). The quality of both biomass and frass as bioconversion products of BSFL degradation is highly influenced by the nutritional composition of the rearing substrate, particularly the balance of macronutrients such as proteins, carbohydrates, and fibers (Siddiqui et al., 2022). Our organic waste facilities have also found that the physical form of BSFL varied with the type of waste used to feed the larvae, further confirming that the type of organic waste stimulates different larval metabolism. This first-hand observation reinforced the view that substrate profile strongly shapes BSFL metabolism, potentially affecting the nutrient, physical form, and overall bioconversion performance of larvae. Understanding how a given organic-waste stream drives the developmental and metabolic fate of BSFL is therefore not merely of biological interest. It provides practical information for designing and managing substrate-specific bioconversion in future waste management systems.

Building on this issue, literature reviews have pursued two largely separate directions. First, the research focused on engineering and optimizing the bioconversion process to enhance biodegradation efficiency and improve the quality of downstream products by combining or adding functional additives, such as antioxidants, to produce value-added materials with enhanced functional properties (Navajas-Porras et al., 2024). Second, the current studies are also investigating the feeding preferences of BSFL to inform the design of more efficient bioconversion systems. Understanding substrate selectivity enables the development of processes that not only accelerate degradation rates but also yield larvae with improved nutrient composition (Fitriana, 2022). Overall, these advancements highlight the potential of BSFL-based systems as a sustainable solution for organic waste management, while simultaneously producing high-value outputs for applications in agriculture and other bio-based industries. However, these studies have typically relied on mixed or compositionally undefined waste streams, and few have isolated the effect of a single, dominant macronutrient class on larval performance under otherwise comparable conditions (Bredun et al., 2024; Gumbo et al., 2025; Upadhyaya et al., 2026). Critically, where macronutrient effects have been examined, larval development has generally been assessed at a single endpoint, rather than tracked across developmental stages, leaving the trajectory of substrate-driven growth and the stage at which different substrates diverge largely uncharacterized.

A second, largely independent body of work has flagged chitin, the structural polysaccharide of the larval exoskeleton, as a limiting factor for BSFL use in aquaculture feed, since it can reduce nutrient digestibility and depress feed performance. Yet how chitin accumulates as a function of the substrate macronutrient profile and developmental stage, and whether that accumulation translates into measurable effects on fish growth, has been examined in a limited number of studies, especially within a single experimental framework. Thus, the present study links these two disconnected strands. To be more specific, the present study aimed to quantify the bioconversion efficiency of four dominant types of domestic-waste substrates with distinct macronutrient profiles (protein-, carbohydrate-, fiber-, and mixed-dominant). The second goal was to track larval growth, including weight and length, as a function of larval developmental stages across the full cultivation period. Third, the present study aimed to characterize extracted chitin obtained from each cultivation treatment and see if it is different in terms of quantity and quality. Finally, the study will conduct a preliminary catfish (*Clarias batrachus*) feeding trial to assess whether chitin in BSFL-derived feed acts as an antinutritional factor (Eide, 2024). Furthermore, Three main hypotheses were made: (i) substrates dominated by readily assimilable macronutrients (protein and balanced mixed substrates) would support higher bioconversion efficiency and superior larval growth than fiber-dominant substrates (H1); (ii) chitin accumulation would increase with developmental progression and would be greatest in the substrates promoting the fastest development toward the pre-pupal stage (H2); (iii) partial substitution of commercial feed with chitin-bearing BSFL pupae would suppress catfish growth relative to a conventional diet, consistent with a dose-dependent antinutritional effect of chitin (H3). By integrating upstream substrate-driven bioconversion dynamics with downstream feed application, the present study sought to bridge the gap between substrate optimization and feed-quality assessment, as well as to generate design criteria for more efficient and nutritionally viable circular-bioeconomy systems.

MATERIALS AND METHODS

Preparation of black soldier fly larvae

Black soldier fly larvae (BSFL) were obtained from the Circularity Center of Universitas Sumatera Utara, Medan, Indonesia. BSFL were reared from BSF eggs in a hopper-style container under dark conditions to facilitate hatching over several days. After the hatching process, BSFL were transferred to clean bio-ponds and reared for an additional seven days on an organic bran-based diet composed of wheat and maize bran sourced from a local supplier. The feeding rate was maintained at 100 mg/larvae/day on a dry basis, while the moisture content of the feed was controlled within the range of 40–60% (Bajra, 2025). Furthermore, the seven-day-old BSFL were subsequently used for bioconversion experiments, as this stage is considered the minimum age for initiating the primary waste bioconversion phase (Eide, 2024). At the end of the rearing period, BSFL were separated from the residual bran using a 25-mesh sieve and transferred to new bio-ponds for subsequent experimental feeding treatments.

Preparation of substrate for feed treatment

Four types of growth substrates were used in the present study, selected based on their dominant macronutrient compositions: tofu represents the protein-rich substrate, green cabbage represents the fiber-rich substrate, rice represents the carbohydrate-rich substrate, and a mixed substrate consisting of equal proportions of tofu, green cabbage, and rice. The tofu will be assigned as protein (P), green cabbage assigned as fiber-rich substrate (F), leftover rice assigned as carbohydrate-rich substrate (C), and mixed waste assigned as balanced macronutrients (M). All the substrates were obtained as daily waste from local markets and waste collection on the campus. The substrates were used in their fresh form without any pretreatment, such as sterilization or mashing. For green cabbage, the substrates were chopped to a uniform particle size of approximately 1–1.5 cm to facilitate larval feeding and ensure consistency across treatments. Figure 1S shows the substrate preparation conducted in the present study.

The experimental diet

The feeding experiment was conducted in plastic container bio-ponds measuring 21 × 11 × 10 cm, where Figure 2S shows the photograph of the bio-pond used in the present study. Approximately 8 g of 7-day-old BSFL was carefully weighed and assigned to each of the four substrate treatments. A standardized feeding rate equivalent to twice the larvae biomass was applied, with 16 g of substrates provided at the beginning of the feeding experiment. The substrate weight was maintained at a constant level, adjusted to correspond with the projected BSFL biomass over the following three days. Each treatment was performed in triplicate, and fresh substrate was supplied daily for 28 days, which is considered optimal for the transition from BSFL to BSF prepupal stage (Lalander et al., 2019). The effectiveness substrate reduction was assessed qualitatively by visual observation of the residual substrate remaining in each bio-pond before the subsequent feeding cycle, rather than by gravimetric measurement. The experiment was conducted in the Solid Waste Laboratory of the Department of Environmental Engineering, Universitas Sumatera Utara. The experiment was conducted at room temperature and humidity, which were recorded as 28 °C and 65%, respectively.

Several parameters were evaluated during the feeding experiment: larval growth performance (weight and length) and chitin yield. The assessments were conducted at different development stages, which were assessed only on days 7 as the starting day, 14, 21, and 28 as the final stage. On the other hand, the transition was visually confirmed by a color change in the BSFL from light yellow in the initial stage of BSFL growth to brown in the initial pre-pupae stage, and dark brown, which signifies full pupation.

Larval development was evaluated through two parameters: larval weight and larval length. For larval weight, ten larvae were randomly sampled from each treatment and weighed individually using an analytical balance (Mettler Toledo MA104/A). The measurement was performed in three replicates to ensure the consistency and reproducibility of the recorded weights. Larval length was determined using image analysis with ImageJ software. BSFL within the bio-pond were photographed using a digital camera, with a sheet of millimeter graph paper affixed to the adjacent section of the bio-pond to serve as a spatial reference. 30 larvae with a clear form will be calculated as the reference for larval length. Furthermore, this reference allowed ImageJ to be calibrated against a known scale, providing a consistent standard of length for measuring all larvae. An example of this calibration and measurement procedure is presented in Figure 3S, where the millimeter graph paper establishes the dimensional control used to standardize length measurements across all BSFL samples. The detailed measurement setup and calibration of larval development are provided in Tables 1S and 2S.

The chitin quantification method

A gravimetric method reported by Triunfo (2023) was employed to quantify the chitin amount in BSFL with some procedure modifications (Triunfo et al. 2022). The analysis was based on the mass of extracted chitin following deproteinization and demineralization . Approximately 10 g of BSFL on each sampling point were first inactivated in boiling water and subsequently dried in an oven at 105°C for 4 hours. The dried BSFL were weighed and recorded as the initial weight (W_1). The samples were then ground into a fine powder before chemical treatment. Furthermore, deproteinization was carried out by treating the powdered sample with 3.5% NaOH solution (Sigma-Aldrich) at a ratio of 1:10 (w/v), followed by heating at 80°C for 2 hours. The resulting mixture was filtered, and the solid residue was thoroughly washed with distilled water to remove residual alkali. Demineralization was subsequently performed by suspending the residue in 1 M HCl (Sigma-Aldrich) at a ratio of 1:5 (w/v) for 30 min at room temperature. The suspension was then filtered and repeatedly washed with distilled water until a neutral pH was achieved. Furthermore, the final solid product was dried at 105°C overnight and weighed as the final chitin mass (W_2). The solid product was further characterized using Fourier transform infrared (FTIR) spectroscopy to confirm the presence of characteristic functional groups of chitins. The chitin yield (%) was calculated based on the initial and final weights according to the equation reported by Pedrazzani (2023) (Pedrazzani et al., 2024). The full extraction and gravimetry procedure is shown in Supplementary Information (Figure 4S), where the detailed calculation of chitin yield measurement is described in Table 3S.

The anti-nutrient test of chitin from BSFL as fish food on catfish cultivation

A subsequent study was designed to evaluate the potential anti-nutritional effects of the chitin derived from BSFL. A randomized experimental design was employed to assess the effects of combining BSFL with commercially available fish feed, with particular emphasis on the influence of chitin content on fish growth performance. A mixing ratio of 50:50 (w/w) between BSFL and commercial feed was selected based on previous studies indicating this proportion as optimal for evaluating the anti-nutritional effects of chitin, although the specific impact of chitin content on catfish growth has not been comprehensively investigated (Kariuki et al., 2024). Two dietary treatments were prepared: (i) 100% commercial feed as the control, and (ii) a mixture of 50% commercial feed and 50% of BSF pupae. The BSFL pupae used in the anti-nutrient test were harvested at 28 days, corresponding to the stage with the highest chitin content as identified in the preceding experimental diet. The commercial feed consisted of standard pellets with a balanced nutritional composition suitable for catfish cultivation (31–32% of protein, 3–5% of lipid, 4–6% of fiber, 10–13% of ash, and 11–13% of moisture), obtained from a local supplier. For the combined diet, the harvested BSF pupae were ground and thoroughly mixed with the commercial feed at the specified ratio, followed by palletization and oven drying at 55°C to ensure uniformity and stability of the feed (Gebremichael et al., 2023).

The anti-nutrient feeding experiment was conducted using catfish (*Clarias batrachus*) obtained from a local aquaculture farm. The fish had an initial length of 7–9 cm and an average weight of 6.2 ± 0.7 grams, corresponding to an approximate age of one month. The catfish were reared in plastic tanks with dimensions of 50 × 20 × 20 cm, with a stocking density of 10 fish per tank. The cultivation experiment was conducted in the Water Analysis Laboratory, Department of Environmental Engineering, Universitas Sumatra Utara. The fish were maintained indoors under ambient laboratory conditions at approximately 28 °C and 50–55% relative humidity. Each cultivation tank contained approximately 15 L of water and was continuously aerated using a commercial air pump at a flow rate of 20 L/min to maintain adequate dissolved oxygen levels throughout the experiment. No water replacement or treatment was performed during the cultivation period to simulate a static culture system. Water quality parameters such as total suspended solids (TSS), total dissolved solids (TDS), pH, and electrical conductivity (EC) were monitored periodically throughout the experiment, and the results are presented in Table 4S. The result of the water quality parameters on the fish tank with different feeds increased gradually with cultivation time, consistent with the progressive accumulation of metabolic solids in the rearing system. All values remained within acceptable limits for catfish culture and were comparable between the two dietary treatments (Caesar et al., 2021). However, the direct water quality parameter such as ammonia, nitrate, and nitrite was not monitored in the present study. Furthermore, the fish were fed twice daily (morning and afternoon) at a feeding rate of 4% of body weight over a 14-day experimental period. The anti-nutrient effect was assessed based on the growth performance of catfish (weight and length) for every five-day interval (Eggink, 2022).

Statistical analysis

All quantitative data are expressed as mean ± standard deviation. Larval length, weight, and chitin yield were compared among the four substrates at each sampling point using one-way analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test. Catfish growth (length and weight) between the control and BSFP-supplemented groups was compared at each time point using an independent-samples (Welch's) t-test. The relationship between larval weight and chitin yield was assessed by Pearson correlation. All analyses were performed in Python (SciPy and stats models), with statistical significance set at $p < 0.05$. The complete ANOVA and Tukey HSD post-hoc output, together with the catfish t-test results, is provided in the Supplementary Information (Table 6S).

RESULTS AND DISCUSSION

Bioconversion of waste based on macronutrient profile using black soldier fly larvae

The present study investigated the bioconversion efficiency of black soldier fly larvae (*Hermetia illucens*) across four substrates with distinct macronutrient profiles, with particular emphasis on waste reduction and sustainability potential. The study was conducted as part of an ongoing waste management development initiative conducted at Universitas Sumatera Utara, where the organic waste stream is characterized by fluctuating composition depending on activities and residents' consumption patterns. To systematically address the highly fluctuating waste properties, macronutrient profiling was adopted as a framework for categorizing and identifying the daily waste characteristics generated within the waste facilities. This approach enables a more targeted and informed application of bioconversion technologies aligned with the actual waste profile being managed. Furthermore, while BSFL have been widely utilized as a protein source for fish feed for local fish farming, the nutritional properties of the resulting larval biomass are not uniform (Siddiqui et al., 2022). The BSFL nutrient profile is inherently influenced by the composition of the substrate on which the BSFL are reared. Thus, the specific effects of single macronutrient-based substrates on BSFL bioconversion performance and larval properties remain largely unreported in the literature. Therefore, the present study addresses the gap by providing foundational insights into how distinct macronutrient profiles, such as protein, carbohydrate, and fiber-rich substrates, differentially influence BSFL bioconversion efficiency, with implications for both campus waste management and the downstream quality of BSFL biomass destined for aquaculture use.

The result on bioconversion efficiency of four substrates, tofu waste demonstrated the highest bioconversion efficiency, with complete substrate consumption observed within approximately 10 hours of feeding, which is ahead of the subsequent daily feeding cycle. The high bioconversion on tofu waste is consistent with the high protein content of tofu shown in Table 1, which provides readily accessible nitrogen sources essential for larval growth and metabolic activity (Schneider et al., 2025). Several previous studies from the literature have reported similar enhanced larval performance and waste reduction rates on protein-rich substrates, attributing the tofu waste substrate to the higher nutritional value that supports rapid enzymatic digestion and tissue synthesis in BSFL (Lalander et al., 2019; Nguyen et al., 2015). The complete absence of residual tofu substrate suggests near-total assimilation, which holds considerable promise for the management of protein-rich food industry by-products, such as soy processing waste, within circular bioeconomy frameworks.

In contrast, green cabbage, characterized by its high fiber content (5.15%), and elevated moisture (91.23%), exhibited markedly lower bioconversion efficiency, with substantial residual material persisting throughout the cultivation period. This outcome aligns with the established understanding that lignocellulosic and fiber-rich plant materials present significant digestive challenges for BSFL due to their complex structural polysaccharides, including cellulose and hemicellulose, which resist enzymatic breakdown (Cickova et al., 2015). The high moisture content of green cabbage may have further compounded this limitation by diluting the available dry matter and creating suboptimal substrate conditions for larval feeding activity (Bruno et al., 2025). Excessive moisture may also reduce oxygen diffusion and air permeability within the substrate, potentially initiating partial composting conditions and a low-oxygen environment. Organoleptic analysis revealed that the high moisture content of the green cabbage substrate generated a strong off-odor resembling rotten eggs, which is suggestive of anaerobic fermentation during the bioconversion process (Pilarska et al., 2023). The fraction of green cabbage that remained unconsumed by BSFL likely underwent anaerobic degradation, plausibly associated with reduced oxygen availability and poor air permeability arising from the formation of dense granular clusters from frass and fine substrate particles, which limit the pore spaces and restrict air circulation within the substrate. However, the effect of oxygen and poor air permeability effect are inferred from the observed moisture, odor, and frass-clustering behavior, rather than from direct measurement. The formation of a granular cluster also restricted BSFL movement, as the larvae tended to adhere to the substrate and were unable to move easily between different regions in the bio-ponds. This reduced mobility limited their feeding activity, causing the BSFL to remain localized rather than actively exploring and consuming the substrate, thereby reducing overall bioconversion efficiency. These findings are consistent with the result reported by El-byari (2025) who found moisture content on the waste negatively affects BSFL performance due to poor aeration and determines the bioconversion efficiency. These findings suggest that fiber-rich vegetable waste, while abundant in urban food waste streams, requires pre-treatment strategies, such as mechanical shredding, fermentation, or blending with nutrient-dense substrates, to enhance its suitability as a BSFL feed substrate.

The role of moisture in bioconversion rate could be well explained in the bioconversion of leftover rice, where the bioconversion process faces optimum and excessive dryness on the leftover rice substrate. In the beginning, the bioconversion process of leftover rice showed rapid degradation due to its soft texture and relatively high moisture content. Nevertheless, the substrate was prone to rapid moisture loss due to its physical characteristics of rice, leading to reduced digestibility over time. Moisture depletion occurs after 12 hours of feeding treatment in bio-ponds exposed to direct sunlight, which causes the rice substrate to dry and harden. This condition kept the leftover rice well-moistened to ensure the complete bioconversion process before transforming into dry, hard biowaste. Excessive dryness in leftover rice limits bioconversion by BSFL since larvae usually chew the waste and convert it into a consumable, slurry-like liquid which depends on the moisture of waste (Salam et al., 2022). As a result, the remaining substrate quantity was significantly lower compared to that of green cabbage, as the least efficiency of bioconversion in the present study, reflecting rapid initial consumption followed by reduced degradation efficiency in the dry substrate.

Furthermore, the mixed waste substrate, with an equal ratio of tofu, green cabbage, and leftover rice, was designed to simulate a balanced macronutrient profile representative of typical household organic waste. The mixed substrate shows an ideal condition that could fulfill the limitation of each substrate. To be more specific, the high moisture content of green cabbage compensated for the rapid drying of leftover rice, while the rice substrate helped balance excessive moisture in the mixed substrate system. This interaction enables the mixed substrate to maintain a more stable moisture level, resulting in improved degradation performance compared to a single-based substrate. The enhanced performance can be attributed to moisture exchange materials, such as green cabbage, and drier components like rice. The improvement in moisture balance supports sustained larval activity and feeding behaviors, ultimately enhancing bioconversion efficiency. Based on the leftover substrate at the end of bioconversion, the green cabbage and leftover rice substrates in the mixed substrate bio-pond have a better bioconversion efficiency compared to a single-based substrate system. The mixed waste reflects real-world municipal food waste composition and offers a practically relevant scenario for large-scale bioconversion applications. The inclusion of leftover rice is particularly noteworthy as carbohydrates serve as a primary energy source for larval metabolic processes and may complement protein-derived nitrogen to optimize growth and waste reduction synergistically. Furthermore, the formation of frass in relatively dry substrates appears to facilitate bioconversion performance since the presence of good frass could facilitate a higher bioconversion rate reported by Lopes (2024). The substrate exhibited moderate moisture content and produced relatively fine and loose frass particles, which enhanced substrate porosity and supported larval movement throughout the bioconversion unit. The obtained condition enables BSFL to actively explore and consume the substrate, thereby improving overall degradation efficiency (Lopes, I.G. et al. 2024). The mixed substrate, therefore represents a promising middle-ground approach, potentially mitigating the limitations associated with mono-substrate feeding while maintaining operationally feasible waste management outcomes.

Based on the bioconversion efficiency investigation, the result further demonstrates that substrate texture and moisture content play critical roles in determining the efficiency of BSFL-mediated waste degradation with the same substrate but different condition. The substrates with high fiber content combined with elevated moisture levels exhibited reduced digestibility, leading to significantly lower bioconversion rates, consistent with previous findings (Peguero et al. 2024). This finding was supported by the bioconversion performance on the mixture substrate, where the green cabbage was left at the end of the bioconversion process, while all other substrates were completely degraded before the following feeding period. The incomplete bioconversion of green cabbage was found at every stage of BSFL growth, including the growth stage at the age of 11-17 days as the optimum degradation phase, indicating the hard bioconversion of high-fiber substrate using BSFL.

Effects of bioconversion and substrate differentiation on larval development

The different efficiencies in the bioconversion rate of BSFL significantly influenced the physical characteristics and growth performance of BSFL, as bioconversion efficiency differentiates the nutrients consumed by BSFL. For larval length development, Table 2 shows the larval body length across the four substrate treatments over the full cultivation period, starting from D-7 (initial feeding, 7-day-old larvae) to D-28 (end of cultivation, 28-day-old larvae). In the beginning, all larvae exhibited a uniform mean length of 4.850 ± 1.144 mm regardless of substrate, which was expected given that all BSFL larvae used in all substrates originated from the same cohort and had not yet been exposed to differential feeding treatments. The uniform baseline for BSFL used in the present study is consistent with the physiological condition of early-instar BSFL, which are characterized by a light body color, soft integument indicating minimal cuticle sclerotization, and active movement, which indicates the healthy development states prior to substrate-driven differentiation (Ipema et al., 2020).

Following the onset of feeding treatment, substrate type exerted a marked and statistically significant effect on larval length development (one-way ANOVA, $p < 0.001$). After seven days of feeding (D-14), larvae reared on the mixed and protein-based substrate achieved the greatest mean lengths, at 12.021 ± 2.495 mm and 10.844 ± 3.023 mm respectively, and were statistically indistinguishable from each other (Tukey HSD, $p>0.05$), which both significantly exceeded larvae reared on leftover rice (9.746 ± 2.436 mm) and green cabbage (5.454 ± 0.968 mm). Relative to the uniform baseline of 4.850 ± 1.144 mm, these values represented length increases of approximately 148%, 124%, 101%, and 12% for the mixed, protein, carbohydrate, and fiber substrates, respectively, with mixed- and protein-fed larvae more than doubling in length within the

Table 1. The nutrient profiles on biowaste substrates in the BSFL cultivation

Substrate	Protein (%)	Carbohydrate (%)	Fiber (%)	Fat (%)	Moisture (%)
Protein from Tofu waste	18.56	5.12	2.32	3.29	72.39
Carbohydrate from Leftover rice	2.72	28.01	0.40	0.05	67.89
Fiber-rich from Green cabbage	1.51	2.02	5.15	0.15	91.23
Balanced Micronutrient from Mixed waste	12.34	17.89	2.24	0.19	76.75

Table 2. Effect of substrate characteristics on the growth (larval length) of black soldier fly larvae at specific cultivation times

Sample	Substrate			
	P (mm)	F (mm)	C (mm)	M (mm)
D-7	4.850 ± 1.144	4.850 ± 1.144	4.850 ± 1.144	4.850 ± 1.144
D-14	10.844 ± 3.023[ab]	5.454 ± 0.968[c]	9.746 ± 2.436[b]	12.021 ± 2.495[a]
D-21	13.700 ± 2.387[b]	8.537 ± 2.357[d]	11.844 ± 1.339[c]	17.194 ± 2.375[a]
D-28	14.837 ± 1.892[b]	11.960 ± 1.638[c]	14.832 ± 2.119[b]	17.299 ± 3.341[a]

first seven days. These early-stage results demonstrate the strong influence of readily assimilable nutrients, particularly protein and balanced macronutrients, in driving rapid larval tissue synthesis, while the fiber-rich cabbage substrate severely constrained early growth. Dietary protein level has been reported to exert a stronger effect on BSFL performance than dietary carbohydrate level, and the present findings corroborate this, with the protein-rich tofu substrate (alongside the protein-containing mixed substrate) producing the most pronounced length increments during the early cultivation stage. Schneider et al. (2025) similarly reported that protein-rich substrates are essential during the early juvenile larval stage to support rapid tissue growth and metabolic activity, while Shah (2024) reports protein-rich feeding as an effective means of enhancing BSFL resistance to bacterial infection.

A statistically significant divergence in growth trajectory became evident from D-21 onward. At D-21, mixed-substrate larvae reached 17.194 ± 2.375 mm, significantly surpassing all other treatments (Tukey HSD, $p < 0.05$), followed by tofu (13.700 ± 2.387 mm), leftover rice (11.844 ± 1.339 mm), and green cabbage (8.537 ± 2.357 mm). At this time point, all four substrates differed significantly from one another. By the final day of cultivation (D-28), the mixed substrate retained the highest mean larval length overall (17.299 ± 3.342 mm), remaining significantly greater than all other treatments, while tofu (14.837 ± 1.892 mm) and leftover rice (14.832 ± 2.119 mm) converged and became statistically indistinguishable from each other, and green cabbage remained significantly the shortest (11.960 ± 1.638 mm). This pattern indicates a transition in nutritional requirements across the larval development stages, from protein-supported demands in early instars to a need for a more complex and balanced macronutrient profile in mid-to-late larval development. Larval weight and length have been shown to vary considerably depending on the feeding substrate, with mixed and more nutritionally diverse substrates generally supporting superior larval development outcomes (Zandi-Sohani and Tomberlin, 2024).

The superior long-term performance of the mixed substrate aligns with the established understanding that BSFL metabolism shifts across developmental stages. During early growth, nitrogen availability from protein drives anabolic processes, whereas the later larval stages increasingly rely on carbohydrates for energy metabolism and fat deposition in preparation for pupation. The mixed substrate, comprising equal proportions of tofu, leftover rice, and green cabbage, provided a complementary macronutrient profile (12.34% protein, 17.89% carbohydrate, 2.24% fiber) that simultaneously met both nitrogen and energy demands across the full cultivation period. Substrate composition exerts a decisive influence on the biochemical properties and development of BSF larvae, confirming their versatility as bioconversion agents. Consistent with this, Addeo et al. (2021) reported that while high-protein substrates promoted rapid larval length increase during early growth, mixed substrates providing a more balanced nutrient composition supported sustained development throughout the cultivation period – a pattern that closely matches the trajectory observed in the present study.

The green cabbage substrate consistently exhibited the lowest growth performance across all time points, reflecting the combined effects of its high fiber content (5.15%), low protein (1.51%), and elevated moisture (91.23%), all of which limit nutrient bioavailability and digestive efficiency in BSFL. The substrates with high lignin and cellulose contents have demonstrated poor digestibility by BSFL, and the structural complexity of the cabbage cell wall likely restricted larval access to assimilable nutrients throughout the cultivation period. Despite this, a progressive, albeit slow, increase in larval length was observed in the green cabbage treatment, from 5.454 ± 0.968 mm at D-14 to 11.960 ± 1.638 mm at D-28, suggesting that BSFL retained a baseline capacity for growth even on nutritionally suboptimal substrates, consistent with their known adaptability to diverse organic waste streams.

Leftover rice, as a carbohydrate-rich substrate (28.01% carbohydrate, 2.72% protein), supported moderate larval growth, ultimately converging with tofu-fed larvae at D-28 (14.832 ± 2.119 mm vs. 14.837 ± 1.892 mm; statistically indistinguishable, Tukey HSD, $p > 0.05$). This convergence suggests that while protein drives early-stage growth velocity, carbohydrate energy sufficiency may partially compensate for lower protein availability over an extended cultivation period, particularly as larvae accumulate energy reserves for the pre-pupal transition.

Taken together, the larval length data demonstrate that substrate macronutrient composition is a critical determinant of BSFL growth trajectory, with distinct nutritional demands characterizing early versus late larval development stages. The mixed substrate emerged as the optimal formulation for sustained growth across the full 21-day feeding period, supporting its potential as the preferred substrate design for BSFL-based bioconversion systems where larval biomass yield is a primary objective. Table 1S shows all the observed data on the length for each BSLF.

The investigation was further conducted by evaluating BSFL weight, as shown in Table 3, providing a complementary dimension to the length data discussed in the preceding section. Larval weight was determined using ten randomly selected individuals per measurement, with each measurement repeated three times to ensure reliability. Table 2S shows the detailed replication data to measure the BSFL weight. On D-7, BSFL weights were uniform across all treatments (0.037 ± 0.002 g per 10 larvae), as the larvae originated from the same cohort and had not yet been exposed to differential feeding conditions. From D-14 onward, all larvae gained weight progressively, although significant divergence among substrates emerged later than it did for larval length.

At D-14, larval weight did not differ significantly among substrate treatments (one-way ANOVA, $p = 0.49$), with all groups recording statistically equivalent values (protein 0.078 ± 0.004 g, fiber 0.080 ± 0.004 g, carbohydrate 0.083 ± 0.004 g, mixed 0.079 ± 0.004 g; all sharing letter "a" in Table 3). This contrasts sharply with the larval length data, which already showed significant substrate-driven divergence at the same time point. The dissociation between length and weight during early development indicates that, while substrate macronutrient profile rapidly influenced longitudinal growth, it had not yet produced measurable differences in body mass. At this stage, the larvae remained soft-bodied with low chitin content (~8–10%; Table 4), so body weight predominantly reflected soft tissue and water content rather than structural chitinous material. These findings are consistent with reports that larval length and weight do not always follow the same substrate-dependent trajectory, as each morphometric parameter reflects distinct aspects of larval physiology and nutrient partitioning (Eggink, 2022; Rasdi et al., 2022).

From D-21 onward, substrate-driven weight differences became pronounced and statistically significant ($p < 0.001$). Tofu- and mixed-substrate larvae diverged markedly from the carbohydrate and fiber treatments, recording the highest weights at D-21 (mixed 0.221 ± 0.011 g and tofu 0.210 ± 0.011 g, statistically indistinguishable from each other and both sharing letter "a"), compared with carbohydrate (0.136 ± 0.007 g) and fiber (0.121 ± 0.006 g), which formed a significantly lighter group (letter "b"). Relative to the D-7 baseline, this represented weight increases of approximately 497% and 468% for mixed and tofu larvae, versus 268% and 227% for carbohydrate and fiber larvae, respectively. This accelerated late-stage weight gain coincided with the onset of the prepupal stage, during which larvae deposit a thickened, chitin-rich cuticle that contributes substantially to body mass (Liu et al., 2023).

Notably, the timing of weight divergence closely tracked chitin accumulation (Table 4). In contrast, chitin content remained low across all substrates at D-14 (~8–10%), coinciding with statistically uniform body weight, both chitin yield and larval weight diverged sharply and in parallel from D-21 onward. The between-substrate coefficient of variation in weight rose from 2.7% at D-14 to approximately 30% at D-21, mirroring the increase in chitin variability. Across the dataset, larval weight and chitin yield were strongly positively correlated (Pearson $r = 0.88$, $p < 0.001$), with the association strongest at D-21 (within-stage $r = 0.96$, $p < 0.05$). These results indicate that progressive chitin deposition in the maturing cuticle is a major contributor to the

Table 3. BSFL weight in relation to substrate characteristics over the cultivation period

Sample	Substrate			
	P (gr)	F (gr)	C (gr)	M (gr)
D-7	0.037 ± 0.002	0.037 ± 0.002	0.037 ± 0.002	0.037 ± 0.002
D-14	0.078 ± 0.004[a]	0.080 ± 0.004[a]	0.083 ± 0.004[a]	0.079 ± 0.004[a]
D-21	0.210 ± 0.011[a]	0.121 ± 0.006[b]	0.136 ± 0.007[b]	0.221 ± 0.011[a]
D-28	0.340 ± 0.017 [a]	0.172 ± 0.009[c]	0.301 ± 0.015[b]	0.316 ± 0.016[ab]

substrate-dependent weight gain observed in later development, consistent with the soft-bodied, low-chitin condition of early larvae in which body mass reflects soft-tissue accumulation rather than structural material.

By the final harvest at D-28, tofu-fed larvae achieved the highest weight at 0.340 ± 0.017 g, followed by mixed substrate (0.316 ± 0.016 g), leftover rice (0.301 ± 0.015 g), and green cabbage (0.172 ± 0.009 g). Tofu and mixed substrates were statistically indistinguishable (both sharing letter "a"), while green cabbage remained significantly the lowest (letter "c"; Tukey HSD, $p < 0.05$). The late-stage convergence between leftover rice and mixed substrate in weight, despite their divergence in length, supports the view that carbohydrate energy sufficiency can partially compensate for lower protein availability in driving mass accumulation over an extended cultivation period, particularly as larvae enter the pre-pupal transition. In contrast, the persistently low weight of green cabbage larvae is consistent with delayed development and the failure of these individuals to transition to the chitin-rich pre-pupal stage, in line with the high moisture content of the substrate (91.23%) and poor nutrient bioavailability (Carpentier et al., 2024). One-way ANOVA confirmed that substrate type significantly affected larval weight from D-21 onward ($p < 0.001$), whereas no significant difference was detected at D-14 ($p = 0.49$).

Substrate-driven larval coloration, cuticle sclerotization, and chitin accumulation

The effect of substrate on BSFL development was further examined by jointly considering larval coloration (Figure 1) and chitin yield (Table 4), which are both complementary indicators, one qualitative and one quantitative, of the same underlying process of cuticle sclerotization and prepupal transition. Larval body coloration served as a qualitative marker of developmental-stage advancement and cuticle melanization, while gravimetric chitin yield provided a quantitative measure of structural cuticle deposition, together complementing the length and weight data discussed in the preceding sections. Morphological progression was documented across all four substrate treatments at D-7 (first day of feed treatment), D-14, D-21, and D-28, the same intervals at which chitin was extracted and quantified.

At D-7, all larvae exhibited a uniform pale pinkish-cream coloration regardless of substrate, consistent with the early-instar physiological condition prior to substrate-driven differentiation. This visual uniformity was mirrored quantitatively by an identical chitin content of 6.975 ± 0.950% across all treatment groups, confirming that cuticle development had commenced within the primary cuticular layer, but had not yet undergone substrate-driven divergence (Soetemans et al., 2020). The soft, lightly pigmented body form at this stage reflects minimal sclerotization and provides a reliable common baseline from which subsequent divergence could be assessed.

By D-14, clear divergence in both coloration and chitin yield emerged among treatments. The tofu-fed larvae displayed a uniform pinkish-salmon coloration with visibly plump, well-defined bodies reflecting active protein-driven tissue growth, and the tofu bio-pond showed a complete absence of residual substrate, consistent with the rapid bioconversion documented earlier. In contrast, the green cabbage-fed larvae appeared darker with a reddish-brown hue, likely influenced by the absorption of chlorophyll degradation products and oxidized plant pigments from the high-moisture substrate rather than intrinsic cuticle melanization, with substantial residual substrate still visible. The leftover rice-fed larvae displayed a distinctively pale white-to-cream coloration, difficult to distinguish from the white rice residue, attributable to the low pigment content of the starch-dominant diet and insufficient precursors for melanin synthesis. Mixed substrate larvae showed

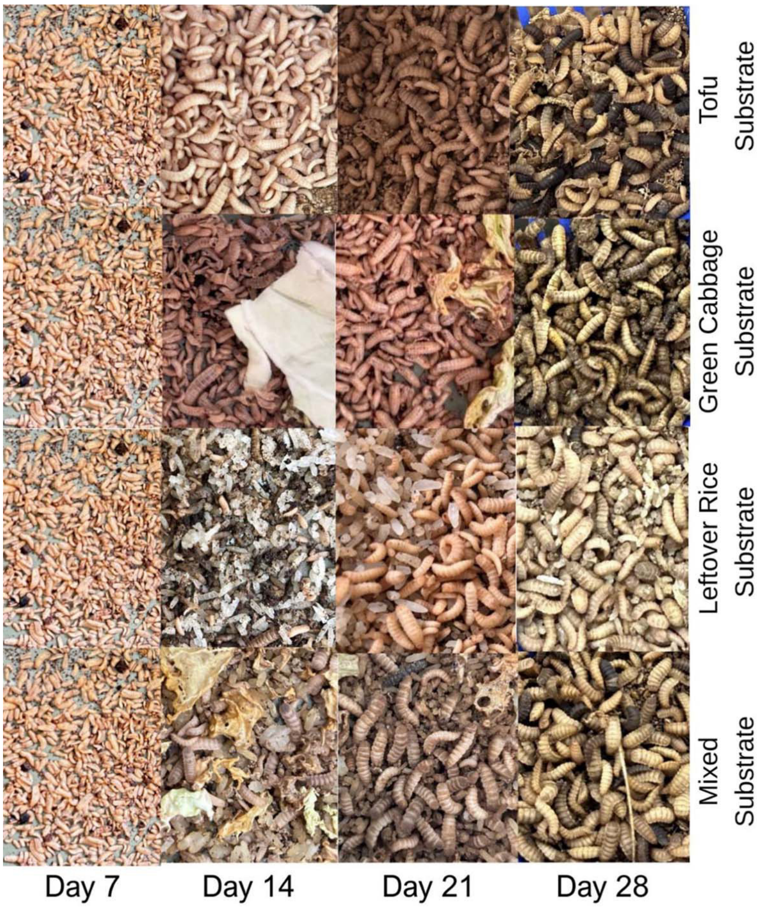

Figure 1. Black soldier fly larvae transformation over time reared at different feed substrates

an intermediate pinkish-brown coloration. These qualitative differences were paralleled by the chitin data. At D-14, the mixed and protein substrates already recorded significantly higher chitin yields (10.306 ± 0.488% and 9.775 ± 0.414%, respectively; sharing letter "a") than the carbohydrate and fiber substrates (8.537 ± 0.508% and 8.395 ± 0.240%; letter "b"; one-way ANOVA, $p = 0.001$). This early chitin elevation indicates that balanced and protein-rich macronutrient provision – combining protein for the chitin–protein matrix with carbohydrate for energy-intensive cuticle biosynthesis – supported early exoskeleton development more effectively than fiber- or carbohydrate-dominant diets (Yang et al., 2021).

Consistent with this, the first pre-pupal individuals were physically observed in the tofu and mixed substrate bio-ponds as early as D-14, although they were not clearly captured photographically owing to their small size at this stage. Pre-pupae in *Hermetia illucens* are identified by a reduction in larval length together with the degree of cuticle pigmentation and sclerotization, and the early appearance of these indicators in the protein-rich and balanced treatments supports the view that nutritional quality is a primary driver of developmental timing in BSFL (Nasution et al., 2026). Because pre-pupal cuticle development is inherently chitin-intensive, the emergence of these individuals directly accounts for the elevated chitin yields recorded in the tofu and mixed treatments at D-14, and in turn for the late-stage larval weight gains described earlier (Albalawneh et al., 2026; Shao et al., 2024).

At D-21, progressive cuticle darkening was evident across all treatments, accompanied by a substantial increase in chitin yield, though both varied markedly by substrate. Tofu-fed larvae exhibited the most advanced medium-to-dark brown coloration with visibly enlarged bodies, consistent with their superior weight gain and accelerating pre-pupal progression, with most pre-pupal individuals observed by D-21. This visual hardening reflects deposition of structural material in the cuticle; calcium carbonate, present in amorphous form in young instars, accumulates as calcite during development, contributing to progressive cuticle hardening alongside chitin (Rebora et al., 2023). The chitin data quantify this divergence: at D-21 all four substrates differed significantly from one another ($p < 0.001$), with the mixed substrate highest (17.048 ± 0.837%; "a"), followed by tofu (14.771 ± 0.429%; "b"), leftover rice (12.304 ± 0.252%; "c"), and green cabbage (10.863 ± 0.317%; "d"). The mixed-substrate larvae showed consistent medium-brown coloration with increasingly frequent pre-pupae, indicating coordinated developmental progression. The leftover rice-fed larvae retained a lighter coloration, reflecting slower progression and reduced precursor availability under carbohydrate-dominant conditions, with a substantial rise in pre-pupal individuals observed only later. The green cabbage-fed larvae showed the least advanced coloration and the lowest chitin yield, consistent with their cumulative nutritional deficiencies. The pronounced chitin accumulation in the mixed and tofu treatments between D-14 and D-21 aligns with the accelerated pre-pupal transition in these groups, as the formation of the thickened, sclerotized pre-pupal cuticle represents the most chitin-intensive event in the BSFL developmental cycle; larval developmental stage, more than substrate alone, is the primary driver of chitin yield, while substrate macronutrient composition modulates its rate and magnitude (Albalawneh et al., 2026).

The most diagnostically significant observations were recorded at D-28. Tofu-fed larvae showed the most intense and widespread black coloration, with the highest proportion of individuals having transitioned to the pre-pupal stage; larval color turns black-gray as BSFL reach the pre-pupal stage, and melanin synthesis is most intensive in the final pre-pupal phase (Ushakova et al., 2018; Chen et al., 2025). Mixed substrate larvae also exhibited black pre-pupae, though at a lower intensity and proportion than tofu. At the quantitative level, chitin yields at D-28 were highest and statistically equivalent in the mixed and tofu treatments (18.136 ± 0.941% and 17.219 ± 0.530%; both "a"), and significantly higher than in the leftover rice and green cabbage treatments (13.101 ± 1.126% and 12.362 ± 0.582%; both "b"; $p < 0.001$). The convergence of the deepest coloration, the highest pre-pupal proportions, and the greatest chitin yields in the tofu and mixed treatments confirms that these substrates most effectively drove the chitin-intensive pre-pupal transition. Notably, although the tofu-fed larvae recorded the highest body weight throughout cultivation, the mixed substrate marginally surpassed tofu in chitin yield at D-28, indicating a subtle divergence between biomass accumulation (favored by protein dominance) and cuticle biosynthesis (favored by balanced macronutrients). The leftover rice-fed larvae showed only the onset of pre-pupal formation at D-28, with the characteristic white-to-cream coloration persisting even into the pre-pupal stage, reflecting reduced melanin precursor availability, as dietary protein and specific amino acids – particularly tyrosine – serve as essential precursors in the melanin biosynthesis pathway (Yakti et al., 2023); their correspondingly low chitin yield is consistent with incomplete metamorphic progression under this unbalanced condition (D'Hondt et al., 2020).

In stark contrast, the green cabbage-fed larvae showed no formation of black pre-pupal individuals by D-28, the only treatment in which the pre-pupal transition was not achieved within the cultivation period; only a small number of individuals had transformed into pre-pupae even by the final day. The persistent absence of the morphological indicators of pre-pupation, together with the lowest terminal chitin yield (12.362%), confirms that these individuals had not reached the developmental threshold required to initiate the pre-pupal transition. Collectively, the parallel trends in coloration, sclerotization, and chitin accumulation demonstrate that substrate characteristics strongly govern BSFL developmental timing, with nutritionally balanced or protein-rich substrates accelerating both metamorphosis and chitin deposition, whereas fiber-rich, poorly degradable formulations prolong the larval stage and delay both pigmentation and chitin-intensive pre-pupal transformation (Kluber et al., 2024).

The transition from the larval to pre-pupal stage presents both advantages and disadvantages for BSFL-based waste bioconversion systems that warrant careful consideration. From a waste-degradation perspective, the onset of the pre-pupal stage marks a critical decline in biodegradation performance, as this stage is characterized by the cessation of feeding while larvae prepare for pupation (Gamero-Barraza et al., 2022). An effective bioconversion process ideally requires larvae to maintain a prolonged active feeding period to extend waste-degradation efficiency, while simultaneously achieving sufficient biomass accumulation for downstream utilization. In the present study, the BSFL reared on green cabbage exhibited the most delayed pre-pupal transition and thus a technically longer active period; however, this was accompanied by consistently ineffective biowaste degradation, as evidenced by persistent residual substrate and the lowest length and weight performance across all treatments. This demonstrates that delayed pre-pupal transition alone confers no bioconversion advantage when the substrate itself limits feeding efficiency. Conversely, the mixed substrate enabled rapid waste reduction through improved nutrient balance and high bioconversion efficiency, but its accelerated pre-pupal transition shortened the effective biodegradation window, indicating that the optimal bioconversion period under this formulation is confined to a limited cultivation timeframe. Although mixed substrates offer fast degradation, the shortened larval feeding duration means harvest timing must be carefully optimized to maximize both waste-reduction efficiency and larval biomass yield within this window (Table 5).

Having established the substrate- and stage-dependent pattern of chitin yield, FTIR spectroscopy was used to verify the identity and structural quality of the extracted chitin (Figure 2). Each FTIR spectrum is shown in Figure 5S. Figure 2(A) presents the spectrum of chitin extracted at D-7, serving as the control before differential feeding. The spectrum displayed the characteristic absorption bands of chitin: a C=O stretching vibration of the Amide I band at 1645.28 cm^{-1} arising from the peptide linkage, and an N–H bending vibration of the Amide II band at 1562.34 cm^{-1}, the positions of which are consistent with α-chitin as previously reported (Gonzalez-Lara et al., 2025; Zlotko et al., 2021). A broad band at 3200–3500 cm^{-1} was attributed to overlapping O–H and N–H stretching associated with extensive hydrogen bonding in the polymer; aliphatic C–H stretching appeared near 2920 cm^{-1}; a peak near 1375 cm^{-1} corresponded to CH_3 deformation; and strong absorption in the 1020–1150 cm^{-1} region was assigned to C–O–C stretching of the glycosidic linkages. The presence of well-defined Amide I and Amide II bands confirms the N-acetyl groups of the N-acetylglucosamine unit, verifying the acetylated polysaccharide framework characteristic of α-chitin (Zlotko et al., 2021; Wang et al., 2020).

Table 4. Quantification of chitin yield from black soldier fly larvae (BSFL) using extraction and gravimetric methods

Sample	Substrate			
	P (%)	F (%)	C (%)	M (%)
D-7	6.975 ± 0.95	6.975 ± 0.95	6.975 ± 0.95	6.975 ± 0.95
D-14	9.775 ± 0.414[a]	8.395 ± 0.240[b]	8.537 ± 0.508[b]	10.306 ± 0.488[a]
D-21	14.771 ± 0.429[b]	10.863 ± 0.317[d]	12.304 ± 0.251[c]	17.048 ± 0.837[a]
D-28	17.219 ± 0.530[a]	12.362 ± 0.582[b]	13.101 ± 1.126[b]	18.136 ± 0.941[a]

Table 5. Characteristic FTIR absorption wavelengths corresponding to the α-chitin fingerprint identified in chitin extracted from BSFL

Sample	O-H/N-H	C-H	Amide I	Amide II	CH_3	C-O-C Glycosidic
D-7	3412	2918	1645.3	1562.3	1379.1	1070/1026
D-14-P	3472/3256	2922	1643.4	1556.6	1379.1	1069/1028
D-14-F	3435/3262	2926	1645.3	1560.4	1381	1072/1024
D-14-C	3441/3262	2926	1639.5	1545	1379.1	1070/1022
D-14-M	3431	2886	1647.2	1545	1379.1	1086/1024
D-21-P	3447	2949/2855	1641.4	1556.6	1381	1070/1022
D-21-F	3408	2926	1647.2	1560.4	1379.1	1067/1024
D-21-C	3433/3269	2922	1649.1/1633.7	1546.9	1379.1	1074/1028
D-21-M	3472/3289	2926	1643.4	1562.3	1379.1	1086/1036
D-28-P	3431	2926	1635.6	1546.9	1379.1	-/1022
D-28-F	3447	2924/2857	1641.4	1572	1381	-/1022
D-28-C	3433	2929	1641.4	1556.6	1386.8	1119/1018
D-28-M	3431/3269	2926	1653	1560.4	1379.1	1080/1032

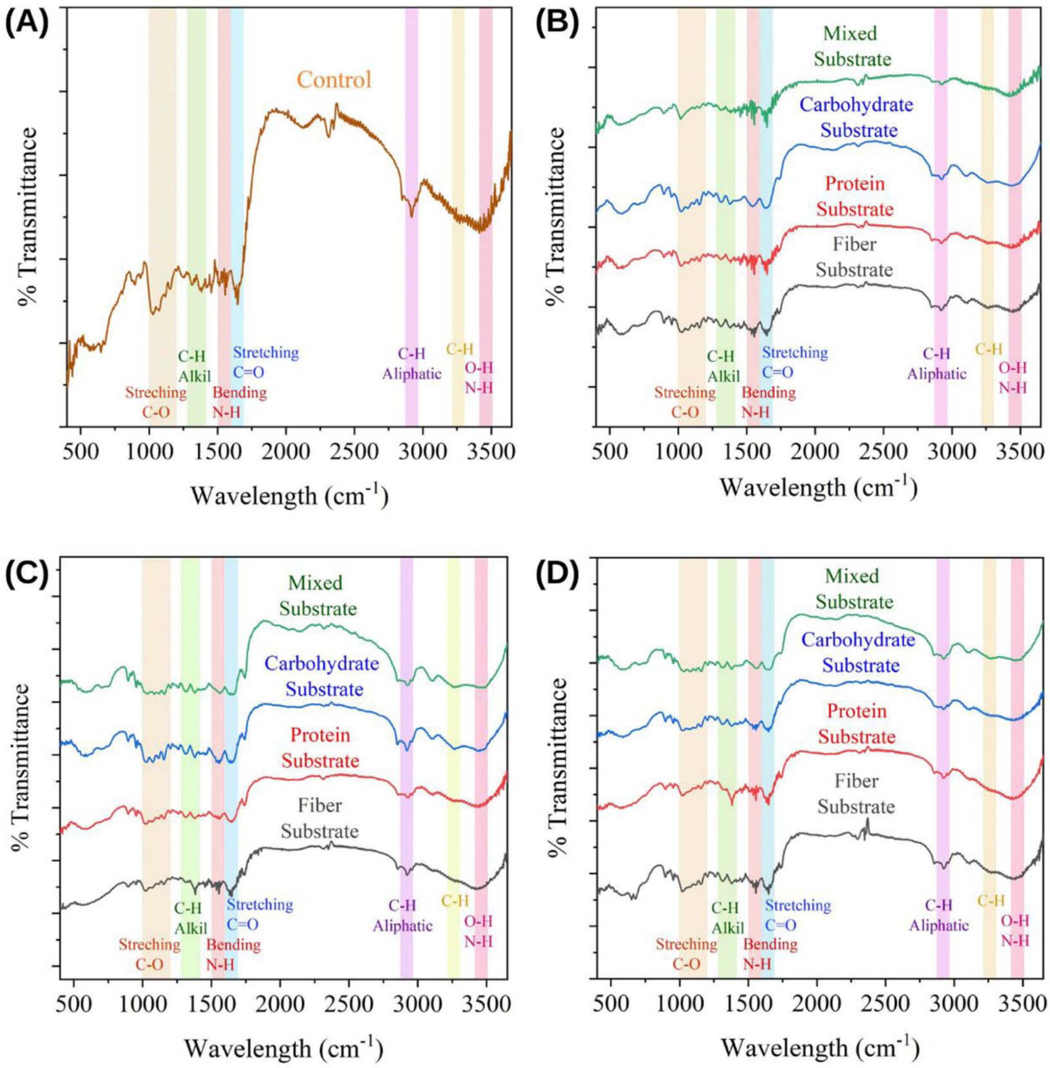

Figure 2. Fourier transform infrared (FTIR) spectra of chitin extracted from black soldier fly larvae: (A) day 7 (control); (B) day 14 (larval stage); (C) day 21 (early pre-pupal stage); and (D) day 28 (pupal stage, final cultivation period).

FTIR spectra were subsequently obtained for chitin extracted at D-14, D-21, and D-28 across all four substrate treatments (Figure 2B–D). The spectra displayed remarkably consistent absorption patterns across all substrates and sampling points, indicating that neither substrate type nor developmental stage altered the fundamental chemical structure of the extracted chitin. The comparable spectral intensities further indicate consistent chitin quality regardless of substrate origin, while the absence of additional bands associated with proteins, lipids, or lignin confirms effective removal of non-chitin components during extraction and the production of structurally consistent, relatively high-purity chitin from all treatment groups. These observations support a critical and practically significant conclusion: substrate macronutrient composition influences chitin quantity but not its quality. While protein-rich and balanced substrates promoted greater chitin accumulation through accelerated pre-pupal development, the structural integrity, degree of acetylation, and molecular framework of the resulting chitin remained consistent across all substrates and developmental stages. This substrate-independent quality consistency highlights the potential of BSFL-based bioconversion for sustainable biopolymer production, whereby diverse organic-waste substrates can be converted into structurally uniform chitin regardless of macronutrient profile – a finding of direct relevance for the standardization and valorization of BSFL-derived chitin in industrial and aquaculture applications.

Preliminary assessment of chitin antinutritional effect on catfish growth performance

The study further investigated the antinutritional effect of chitin contained in BSFL pupae on the growth performance of catfish (*Clarias batrachus*). BSFL pupae are widely utilized as a protein-rich feed ingredient in aquaculture and are commonly incorporated into fish diets in Indonesia. Nevertheless, the presence of chitin in insect-based feed has been reported to exert antinutritional effects, potentially limiting nutrient digestibility and impairing growth performance (Eggink et al., 2022; Wang et al., 2020). Despite these findings, comprehensive studies on the antinutritional impact of chitin specifically on catfish (*Clarias batrachus*) remain limited – a gap of particular relevance given that catfish represent one of the most extensively cultivated freshwater species in Indonesia (Deswati et al., 2025). Since FTIR analysis confirmed that substrate macronutrient composition influences chitin quantity, but not chitin quality, with all substrates producing structurally identical α-chitin, BSFL pupae randomly harvested from the mixed and tofu substrate bio-ponds were combined and incorporated into the experimental diet without substrate-based differentiation. The present section, therefore, aimed to evaluate the antinutritional effects of the chitin derived from BSFL pupae on catfish growth performance, providing foundational evidence for evidence-based feeding strategies in Indonesian catfish aquaculture.

Two treatment groups were established: a control group fed 100% CFF, and a treatment group fed a composite diet of 50% CFF supplemented with 50% BSFL pupae meal (CFF:BSFP). Catfish growth was evaluated using five randomly selected fish per tank, with five replicates, to ensure data reliability. Fish were fed twice daily at 4% body weight over a 14-day period, with measurements recorded at five-day intervals. Initial individual weights were 6.6 ± 0.3 g (CFF) and 6.2 ± 0.7 g (CFF:BSFP), and initial lengths were 8.7 ± 0.1 cm (CFF) and 8.5 ± 0.3 cm (CFF:BSFP), confirming comparable baseline conditions prior to dietary differentiation. Growth differences between the two groups were assessed at each time point using an independent-samples (Welch's) t-test, with significance set at $p < 0.05$.

Figure 3(A) presents body length across the experimental period. At D-0 and D-5, the two groups were statistically comparable (8.7 ± 0.1 vs. 8.5 ± 0.3 cm at D-0, $p = 0.18$; 9.0 ± 0.4 vs. 8.7 ± 0.3 cm at D-5, $p = 0.25$), reflecting a period in which fish grew only slightly regardless of dietary treatment. A pronounced divergence became evident by D-10, where the CFF fish increased in length by approximately 25% relative to D-5, compared with only about 10% in the CFF:BSFP group; this difference was statistically significant (11.7 ± 0.5 vs. 9.8 ± 0.4 cm, $p < 0.001$). By D-14, control fish reached a mean length of 13.1 ± 0.5 cm compared with 11.7 ± 0.4 cm in the BSFL treatment group ($p = 0.002$). The consistent and significant length advantage of the control group from D-10 onward indicates that the presence of chitin in the composite feed exerted a negative effect on length development, particularly following prolonged dietary exposure. Chitin in BSFL is widely recognized as an antinutritional factor for fish, with several studies reporting reduced feed intake and nutrient digestibility, and the length suppression observed in the CFF:BSFP group from D-10 onward is consistent with this established mechanism.

A more pronounced treatment effect was observed in weight, as presented in Figure 3(B). At D-0 and D-5, individual weights were statistically comparable between groups (6.6 ± 0.3 g vs. 6.2 ± 0.7 g at D-0, $p = 0.22$; 7.5 ± 1.1 g vs. 6.8 ± 0.7 g at D-5, $p = 0.24$). By D-10, a striking and significant divergence emerged: CFF fish recorded a mean individual weight of 17.0 ± 2.4 g compared with only 9.7 ± 1.2 g in the CFF:BSFP group ($p < 0.001$), approximately 75% higher in the control group. This divergence widened further by D-14, where control fish reached 24.0 ± 2.9 g compared with 16.9 ± 2.0 g in the treatment group ($p = 0.003$). These findings confirm that chitin in the composite diet exerted significant antinutritional effects on weight gain, consistent with previous reports across multiple fish species, including Atlantic salmon (Karlsen et al., 2015), rainbow trout (Pascon et al., 2025), and Nile tilapia (Eggink et al., 2022). Chitin increases digesta transit time owing to its viscosity, reducing exposure of digesta to gut enzymes, while also interfering with intestinal proteases and binding lipids to decrease their absorption, collectively impairing the digestion and assimilation of protein, lipid, and energy from the composite diet (Hopkins et al., 2021). Table 5S shows the detailed data of catfish development obtained in the present study.

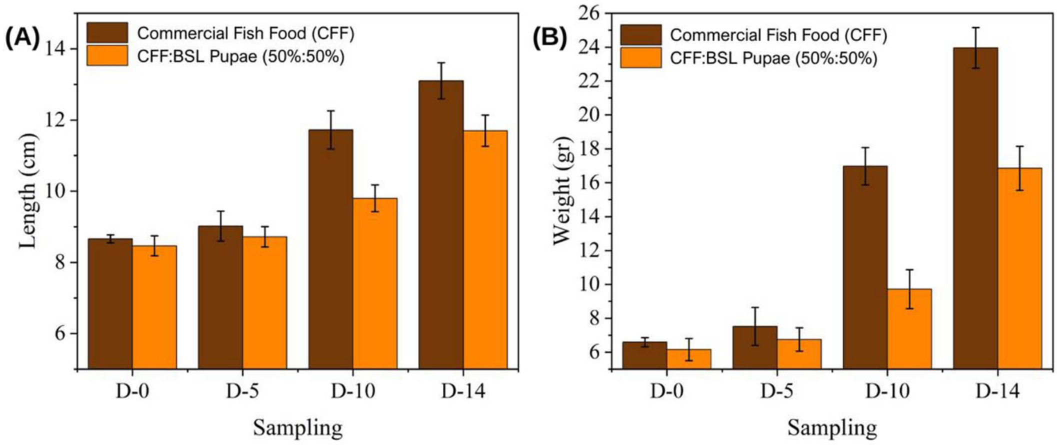

Figure 3. Catfish growth performance based on (A) length and (B) weight under commercial fish feed (CFF) and composite fish feed supplemented with Black Soldier Fly pupae (CFF:BSFP)

Nevertheless, the effects of chitin and its derivatives are not universally detrimental and are highly species- and dose-dependent. Salam et al. (2021) demonstrated that supplementation with low doses of chitosan (0.4–1.0 g/kg feed) enhanced growth and body weight in juvenile silver barb (*Barbonymus gonionotus*), indicating that controlled inclusion levels may provide physiological benefits (Salam et al., 2021). Similarly, Karlsen et al. (2015) reported that chitosan negatively affected Atlantic salmon but showed no measurable impact on Atlantic cod or Atlantic halibut, highlighting a species-dependent response likely associated with differences in digestive capacity and chitinolytic enzyme activity. The presence of chitin can affect the digestibility of feed in some fish species, making the inclusion of insect-based meals in aquafeeds complex, with reduced growth rates observed in certain species while other species may benefit from its prebiotic properties. These contrasting findings across the literature collectively suggest that the antinutritional role of chitin is highly context-dependent, particularly with respect to species, dosage, and feed formulation, and that investigating its effects specifically on catfish is critical for informing sustainable feed development in Indonesian aquaculture.

A targeted mechanistic analysis further clarifies the basis of the observed growth suppression. Naeem et al. (2024) identified an optimal dietary protein requirement of approximately 40% for *Clarias batrachus* to achieve maximal somatic growth (Naeem et al., 2024), and notably, both the CFF and BSFL pupae evaluated in the present study contained the protein levels theoretically sufficient to sustain growth, suggesting that protein availability alone does not explain the observed performance disparity between treatment groups. Instead, the reduced growth is more plausibly attributed to impaired nutrient utilization associated with elevated dietary chitin content. Chitin has been reported to interfere with protein digestibility by accelerating intestinal transit and limiting the exposure of dietary proteins to proteolytic enzymes, thereby constraining hydrolysis and amino acid absorption (Hopkins et al., 2021). From a physiological perspective, this diminished digestive efficiency shifts the feed conversion balance unfavorably, ultimately suppressing growth despite adequate crude protein levels in the diet. Therefore, the present study confirms that the nutritional value of high-protein feed ingredients must be interpreted in the context of their structural composition and digestibility constraints and that the inferior performance observed in catfish fed with CFF:BSFP reflects a digestibility-mediated bottleneck, rather than a deficit in nutrient supply per se.

In the present study, the chitin content in the composite CFF:BSFP diet was estimated at approximately 8–9%, based on the measured pupae chitin yields of 17–18% at D-28 and the 50% BSFL pupae inclusion level. For context, previous studies have reported that dietary chitin inclusion of up to ~3% did not impair growth or nutrient digestibility, whereas inclusion of around 4.5% began to reduce performance; the estimated level in the present diet therefore exceeds these literature-based thresholds, which may, in part, account for the growth suppression observed. However, because the present trial compared a whole-pupae composite feed with a commercial feed and did not isolate chitin from other compositional differences between the diets, these values should be regarded as indicative, rather than definitive. Taken together with the broader literature, the findings tentatively suggest that keeping dietary chitin relatively low may help preserve catfish growth performance while retaining the nutritional benefits of BSFL pupae, although a specific tolerance threshold remains to be established through controlled trials with graded, purified chitin inclusion levels.

It is important to acknowledge several limitations of the present study. First, the antinutritional trial used whole BSFL pupae meal, rather than purified chitin, so the observed growth suppression reflects the combined properties of the pupae matrix and cannot be attributed to chitin alone, independently of other compositional differences between the two diets. Second, the study did not systematically examine the effect of varying chitin inclusion levels and was conducted over a short, 14-day period as an initial assessment of BSFL pupae incorporation in catfish feed; consequently, the precise dose–response relationship between dietary chitin level and growth performance in *Clarias batrachus*, and its longer-term impact, could not be established from the present data alone. Third, chitin was quantified using a published gravimetric protocol without validation against a commercial chitin standard, and the number of replicates (larval weight and chitin, n = 3; fish, n = 5) limits statistical power. Finally, growth was assessed through length and weight only, while the dissolved oxygen, ammonia, and nitrite concentrations and fish health indicators that most directly reflect rearing stress were not measured. Further studies are therefore required to determine the precise chitin tolerance threshold in catfish using longer grow-out trials with graded, purified chitin inclusion levels and additional physiological and health indicators, to evaluate lower BSFL pupae inclusion levels (10–30%), and to investigate pretreatment strategies to reduce chitin-related limitations, including microbial degradation using chitinolytic bacteria, which have shown promise for improving the nutritional quality of BSFL biomass (Ashouri et al., 2026). Feed formulation should also be carefully optimized when incorporating insect-derived protein into aquafeeds to ensure that sustainability gains are not achieved at the expense of biological performance. The present findings nonetheless provide an important preliminary evidence base for the responsible and optimized integration of BSFL-derived ingredients into catfish aquaculture systems in Indonesia.

CONCLUSIONS

The present study demonstrated how the macronutrient profile of biowaste substrates governs BSFL bioconversion, larval development, chitin accumulation, and the downstream suitability of BSFL pupae as catfish feed. The first hypothesis (H1) was supported: the protein-rich (tofu) and balanced mixed substrates achieved the highest bioconversion efficiency and the greatest larval growth, whereas the fiber-rich green cabbage substrate consistently performed the poorest and failed to complete pre-pupal transition within the cultivation window. The second hypothesis (H2) was also supported: chitin yield increased progressively with developmental stage and was greatest in the mixed and tofu substrates that drove the fastest pre-pupal development, while FTIR confirmed that substrate composition altered chitin quantity, but not its α-chitin structure, establishing that diverse organic wastes can be converted into structurally uniform chitin. The third hypothesis (H3) was supported only preliminarily: partial substitution of commercial feed with chitin-bearing BSFL pupae significantly reduced catfish growth over the 14-day trial, consistent with an antinutritional effect, although the present design could not isolate the specific contribution of chitin from other compositional differences between the two diets. The principal new contribution of this work is the integration, within a single experimental framework, of upstream substrate-driven bioconversion and larval development with downstream chitin characterization and a preliminary feed application – a linkage rarely examined together that bridges the gap between substrate optimization and feed-quality assessment. The findings provide practical design criteria for substrate-specific bioconversion in decentralized waste-management systems, indicating that balanced (mixed) substrates offer the best compromise between waste-reduction efficiency and larval biomass yield, provided harvest timing is optimized around the accelerated pre-pupal transition. Because the catfish trial was preliminary and short-term, the observed growth suppression should be regarded as an early indication rather than a definitive dose–response relationship, and a specific dietary chitin threshold cannot be established from the present data. Future work should employ longer grow-out trials with graded, purified chitin inclusion levels, validated chitin quantification, and additional physiological and health indicators to confirm these findings and to determine a reliable chitin tolerance threshold for *Clarias batrachus*.

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