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EFFECTS OF HOUSEHOLD ORGANIC WASTE SUBSTRATES ON BLACK SOLDIER FLY LARVAL PERFORMANCE AND BIOCONVERSION

Mai Van Phong[1], Thai Hong Quynh Chi[1,2], Ngo Phuoc Thao Tran[2], Nguyen An Bao Chau[2], Hoang Anh Tuan[2], Ngo Quang Bao[2]

[1]Polytechnic College of – Engineering and Technology, ThuongTin ward, HaNoi city, VietNam
[1,2]Amsterdam Highschool for the gifted
[2]Wellspring Saigon International Bilingual School
[2]Vinschool Smart City Secondary and High School
[2]Ha Long High School for the gifted
[2] HUS High School for the gifted.

ABSTRACT:
Household organic waste constitutes a substantial fraction of municipal solid waste and requires decentralized treatment solutions adapted to local conditions. This study evaluated the performance of black soldier fly larvae (Hermetia illucens; BSFL) for household-scale organic waste bioconversion in Ninh Binh, Vietnam. A field experiment was conducted across 30 households using three substrate treatments, with 10 independent household replicates per treatment: vegetable and fruit waste (Group A), animal-derived food waste (Group B), and mixed household organic waste (Group C). Initial stocking was standardized at approximately 35,000 eggs per container, with an estimated allocation tolerance of ±500 eggs, and the experiment was conducted over 21 days. Waste reduction efficiency (WR), directly measured total fresh larval biomass, larval body length, and NaOH-extractable soluble protein content were evaluated. Substrate treatment was associated with significant differences in the evaluated performance indicators. Group C achieved the highest WR (83.54 ± 0.79% on a dry-matter basis), followed by Group B (75.41 ± 1.99%) and Group A (63.23 ± 2.11%). In contrast, Group B showed the highest directly measured total fresh larval biomass (439.6 ± 14.4 g/container) and mean larval body length (2.90 ± 0.09 cm) after 21 days. NaOH-extractable soluble protein content, determined using the Bradford assay following extraction with 0.1 M NaOH, was highest in Group B (47.79 ± 1.04% DM), followed by Group C (44.56 ± 0.95% DM) and Group A (39.95 ± 1.03% DM). These findings indicate that substrate treatment was associated with differences in waste reduction and BSFL performance under household-scale conditions. Mixed household organic waste showed the highest waste reduction efficiency, whereas animal-derived food waste was associated with greater fresh larval biomass, body length, and NaOH-extractable soluble protein content. Further multi-season and multi-location studies with standardized dry-matter substrate loading, detailed substrate characterization, and direct quantification of hatching success and final larval abundance are needed to determine the broader applicability of the system.

KEYWORDS: Black soldier fly larvae; Organic waste management; Bioconversion; Household-scale; Soluble protein

Abbreviations (if any): BSFL, black soldier fly larvae; DM, dry matter; WR, waste reduction efficiency; BSA, bovine serum albumin; ANOVA, analysis of variance; HSD, honestly significant difference; SD, standard deviation.
Running title: Household Waste Bioconversion Using BSFL

INTRODUCTION (10 PT)

The rapid increase in household organic waste has become an important environmental challenge in Vietnam, particularly in rapidly urbanizing areas. Previous field studies in Vietnamese cities have shown that biodegradable and food-derived materials constitute a substantial fraction of municipal solid waste. In Hoi An, degradable waste accounted for more than half of municipal waste, while household waste generation also differed significantly between urban and rural areas (Hoang et al. 2017). Similarly, a municipal solid waste characterization study in Da Nang reported that biodegradable waste represented approximately 54% of the total waste stream, with food waste being a major component (Pham Phu et al. 2021). These findings highlight the need for decentralized biological treatment approaches capable of recovering value from the organic fraction of household waste.
Black soldier fly larvae (BSFL), Hermetia illucens, have received increasing attention as a biological approach for converting organic waste into larval biomass and residual material. Previous studies have demonstrated the capacity of BSFL to process a wide range of organic substrates, including food waste, vegetable and fruit residues,

agro-industrial by-products, and mixed organic wastes (Nguyen et al. 2015; Jucker et al. 2017; Chia et al. 2018; da Silva and Hesselberg 2020; Surendra et al. 2020). In addition to reducing organic waste, BSFL bioconversion can generate larval biomass containing substantial amounts of protein and lipids, creating opportunities for resource recovery and circular utilization of organic waste (Tschirner and Simon 2015; Barragán-Fonseca et al. 2017; Spranghers et al. 2017). From an agricultural perspective, the biological conversion of organic residues is particularly relevant to circular resource-use strategies because it links organic waste management with the recovery of biologically derived materials. Household-scale BSFL systems may therefore provide a useful model for evaluating decentralized resource recovery under practical conditions, particularly in areas where organic residues are generated in relatively small and heterogeneous streams.

The performance of BSFL bioconversion depends on both substrate characteristics and rearing conditions. Feeding rate, substrate composition, nutrient availability, moisture content, and larval density have been reported to influence larval development, waste reduction, biomass production, and nutritional composition (Diener et al. 2009; Parra Paz et al. 2015; Barragán-Fonseca et al. 2018; Meneguz et al. 2018; Bekker et al. 2021). In particular, dietary nutrient composition can influence larval development and the protein and lipid composition of harvested biomass (Spranghers et al. 2017; Barragán-Fonseca et al. 2018; Hopkins et al. 2021). However, differences in substrate moisture and dry-matter availability may also affect apparent treatment performance, making it important to consider the basis on which feeding is standardized when comparing heterogeneous organic waste streams.

Although the potential of BSFL technology has been demonstrated in numerous studies, much of the available experimental evidence has been generated under controlled or semi-controlled rearing conditions using defined or standardized organic substrates (Diener et al. 2009; Meneguz et al. 2018; Lalander et al. 2019). Under decentralized household-scale conditions, organic waste composition can vary among households and over time. Such variability, together with natural fluctuations in temperature and relative humidity, may influence larval performance and bioconversion outcomes (da Silva and Hesselberg 2020; Bekker et al. 2021). These conditions differ from standardized laboratory experiments but are directly relevant to practical decentralized organic-waste management and resource-recovery systems. Evaluating BSFL across independent household units can therefore provide complementary evidence on how the system performs under realistic field conditions where some degree of substrate and household-level variability is unavoidable. Field-based evidence evaluating different household-generated organic waste streams under decentralized rearing conditions remains comparatively limited, particularly in Vietnam.

To address this gap, the present study evaluated waste reduction efficiency, total fresh larval biomass, larval body length, and NaOH-extractable soluble protein content of BSFL reared on three household organic waste-stream categories across 30 households in Ninh Binh Province, Vietnam. Based on previous evidence that substrate characteristics are associated with BSFL waste reduction and development (Diener et al. 2009; Barragán-Fonseca et al. 2018; Meneguz et al. 2018), two hypotheses were proposed:

Hypothesis 1 (H_1). Mixed household organic waste will achieve higher waste reduction efficiency than the vegetable-and-fruit and animal-derived food waste treatments under household-scale rearing conditions.

Hypothesis 2 (H_2). Animal-derived food waste will result in greater total fresh larval biomass and larval body length and higher NaOH-extractable soluble protein content than the other substrate treatments.

The findings are expected to provide field-based evidence on BSFL performance across different household organic waste streams and contribute to the development of decentralized organic waste management and agricultural resource-recovery approaches under local conditions.

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted from March to May 2026 in Le Ho Ward, Ninh Binh Province, Vietnam, encompassing the household rearing experiment and subsequent laboratory analyses. The 21-day household rearing experiment was conducted concurrently across all 30 participating households from 20 March to 9 April 2026. All households were located within the same local area. The study area is characterized by a humid subtropical climate typical of northern Vietnam. During the experimental period, temperature ranged from approximately 24.5 to 31.8°C, while relative humidity ranged from 72 to 84%.

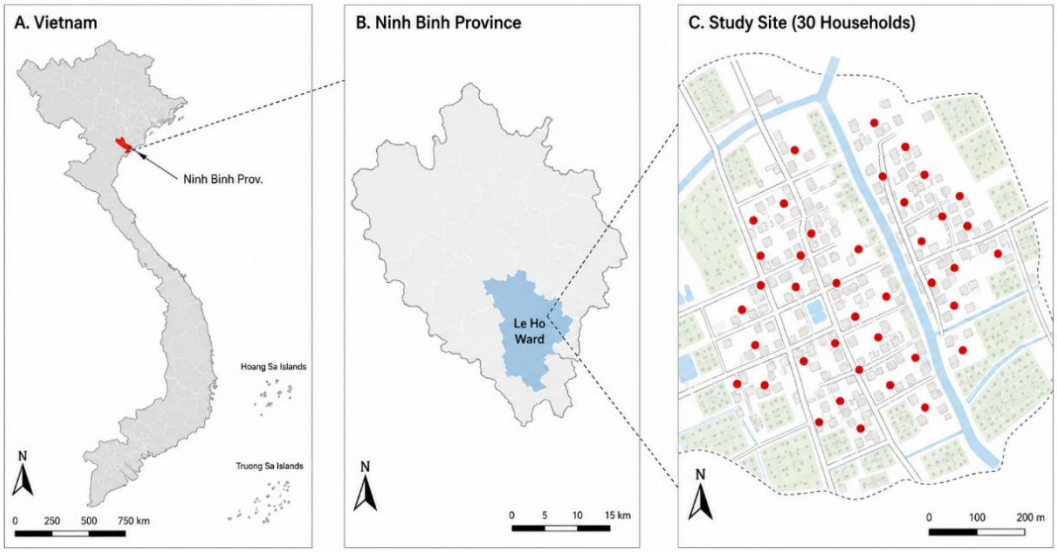

Figure 1. Map of the study area in Ninh Binh Province, Vietnam, indicating the locations of the 30 participating households.

3.2. Experimental Design

The experiment was conducted as a household-based field trial involving 30 participating households within the same local area. Households were organized into three substrate-treatment groups, with 10 independent household containers per treatment (n = 10). The three treatments were: Group A – Vegetable and Fruit Waste (VFW), consisting of discarded vegetables and fruits generated from routine kitchen activities; Group B – Animal-derived Food Waste (AFW), consisting primarily of leftover meat, animal fat, fish viscera, and other animal-derived food scraps; and Group C – Mixed Organic Waste (MOW), consisting of a mixture of vegetable residues and animal-derived food waste generated during routine household kitchen activities.

Each participating household was provided with an identical plastic rearing container measuring 60 × 40 × 30 cm (length × width × height). Containers were fitted with mesh-covered lids to provide ventilation and were maintained under sheltered household conditions to minimize direct sunlight and rainfall. All 30 containers were operated concurrently under the same standardized feeding schedule and monitoring protocol throughout the 21-day rearing period.

To promote protocol consistency across households, three members of the research team were assigned to supervise the three treatment groups, respectively. The assigned researcher visited the participating households daily to guide substrate weighing and feeding, verify adherence to the feeding schedule, and monitor implementation of the experimental protocol.

Figure 2. Representative household-scale BSFL rearing containers and organic waste substrates used in the field experiment.

3.3. Larval Inoculation

Black soldier fly (Hermetia illucens) eggs were obtained from the same local commercial insect-rearing facility to ensure a consistent source across experimental units. Initial stocking was standardized using an egg mass-to-number calibration procedure. For calibration, 500 eggs were manually counted under a stereomicroscope and subsequently weighed. This procedure was independently repeated three times, and the mean mass of 500 eggs was used to establish the relationship between egg mass and egg number.

Based on this calibration, an egg mass corresponding to approximately 35,000 eggs was allocated to each experimental container. An estimated tolerance of approximately ±500 eggs was used to account for uncertainty

associated with mass-based egg allocation. Eggs were placed directly into their respective experimental containers and allowed to hatch in situ before larval development proceeded under the assigned substrate treatment.
Hatching success was not independently quantified, and larvae were not recounted immediately after hatching. Therefore, the estimated initial egg number should not be interpreted as a directly measured initial larval abundance, and subsequent final larval abundance was not used to calculate an absolute survival rate.

3.4. Substrate Preparation and Feeding Regime

Household organic waste was collected daily by the participating households. Prior to feeding, non-organic contaminants, including plastic materials, ceramic fragments, and large animal bones, were manually removed. The organic waste was then manually chopped into pieces of approximately 1–3 cm to improve substrate uniformity before being supplied to the larvae.
All 30 experimental containers followed the same predefined fresh-mass feeding schedule throughout the 21-day rearing period. The feeding amount was gradually increased according to larval development, from 1.0 kg container^{-1} day^{-1} during the initial days to a maximum of 2.5 kg container^{-1} day^{-1} during the later rearing period, with minor reductions during the final two days. The cumulative fresh-substrate input was controlled at 42.0 kg per container for all three treatments. Daily substrate quantities were weighed before feeding, and compliance with the feeding schedule was monitored by the assigned research team member during daily household visits.
Substrate dry-matter (DM) content was determined separately for each household experimental unit on Days 1, 7, 14, and 21 using fresh substrate collected immediately before feeding. A representative subsample was weighed fresh and subsequently oven-dried to constant mass to determine DM content. These repeated household-level measurements showed substantial differences in DM content among the three waste-stream treatments, with VFW containing approximately 9.4–10.4% DM, AFW 24.4–26.0% DM, and MOW 18.8–20.2% DM across the sampling occasions. Consequently, although all treatments received an identical cumulative fresh-substrate mass, their cumulative DM inputs differed.
The experiment was therefore standardized on a fresh-mass basis rather than a dry-matter basis, reflecting the practical handling of household organic waste under field conditions. Household-level DM measurements and daily feeding records were retained to characterize the corresponding DM input for each experimental unit.

3.5. Larval Performance and Waste Reduction Assessment

Larval growth was assessed on Days 7, 14, and 21. At each sampling point, 100 larvae were randomly collected from each experimental container. The body length of each larva was measured individually using a digital caliper, and the 100 larvae were weighed collectively using a laboratory analytical balance. Mean individual fresh mass was calculated by dividing the collective mass by 100. After measurement, the sampled larvae were returned to their respective experimental containers.
At Day 21, all larvae were manually separated from the residual substrate and harvested from each container. The larvae were cleaned to remove adhering substrate and surface material and then air-dried under standardized conditions using a fan in an air-conditioned environment (25°C for 30 min) to remove surface moisture. The entire larval harvest from each container was subsequently weighed using a laboratory analytical balance to obtain the directly measured total fresh larval biomass. Final larval abundance was then estimated for each container by dividing the directly measured total fresh biomass by the mean individual fresh mass determined from the random 100-larva subsample from the same container.
At the end of the 21-day rearing period, the entire recoverable residual substrate remaining in each container was weighed separately on a fresh-mass basis. A representative sample of residual substrate from each container was then oven-dried at 105°C to constant mass to determine its dry-matter content and calculate residual substrate dry mass. Frass was separated from the residual substrate but was not quantified independently. No appreciable leachate or substrate spillage was observed during the experiment.
Waste reduction (WR) was calculated as:

$$\mathrm{WR}(\%) = \frac{\mathrm{DM}_{\mathrm{input}} - \mathrm{DM}_{\mathrm{residue}}}{\mathrm{DM}_{\mathrm{input}}} \times 100$$

where DMinput represents the estimated cumulative dry-matter input supplied to each container during the 21-day rearing period and DMresidue represents the dry mass of the recoverable residual substrate remaining at harvest. Because frass was not independently quantified, WR was interpreted as the reduction in recoverable residual substrate dry matter rather than a complete system-level dry-matter mass balance.

3.6. Determination of NaOH-Extractable Soluble Protein Content

Larval biomass harvested on Day 21 was oven-dried at 60°C to constant weight and ground into a fine powder. For each household container, 0.100 g of dry larval powder was extracted with 10.0 mL of 0.1 M NaOH at room temperature (25 ± 2°C) for 60 min under gentle shaking. The mixture was subsequently centrifuged at 10,000 × g for 15 min at 4°C, and the resulting supernatant was diluted 1:10 (v/v) prior to protein determination.
NaOH-extractable soluble protein was quantified using the Bradford colorimetric assay with Coomassie Brilliant Blue G-250 reagent. Briefly, 20 µL of diluted extract was mixed with 1.0 mL of Bradford reagent in a cuvette, incubated for 5 min at room temperature in the dark, and absorbance was measured at 595 nm using a UV–Vis spectrophotometer. Bovine serum albumin (BSA) was used to construct the calibration curve ($R^2 \geq 0.996$).

Larval samples from each of the 30 household containers were processed and analyzed independently, providing 10 independent biological replicates per treatment, with no pooling among households. Results were expressed as NaOH-extractable soluble protein content on a dry-matter basis (% DM).

3.7. Statistical Analysis

All data collected from the 30 participating households were entered and checked in Microsoft Excel and analyzed using IBM SPSS Statistics version 26.0. Each household rearing container was considered an independent experimental unit, resulting in 10 independent biological replicates per treatment (n = 10).

Data normality and homogeneity of variance were assessed using the Shapiro–Wilk test and Levene's test, respectively. Treatment effects on final Day-21 response variables, including waste reduction efficiency (WR), directly measured total fresh larval biomass, mean individual fresh mass, mean larval body length, and NaOH-extractable soluble protein content, were evaluated using one-way ANOVA, followed by Tukey's HSD test for pairwise comparisons when significant treatment effects were detected.

Larval body length and individual fresh mass measured on Days 7, 14, and 21 were analyzed using a two-factor repeated-measures ANOVA, with rearing time (Day 7, 14, and 21) as the within-subject factor and substrate treatment (VFW, AFW, and MOW) as the between-subject factor. Treatment, time, and treatment × time interaction effects were evaluated.

Results are presented as mean ± standard deviation (SD) unless otherwise stated. Statistical significance was set at $p < 0.05$. For inferential analyses, exact p-values, F statistics, degrees of freedom, and effect sizes were reported where appropriate.

4.RESULTS AND DISCUSSION

A. RESULTS

4.1. Household organic waste reduction performance

Waste reduction differed significantly among the three household waste-stream treatments after the 21-day rearing period (one-way ANOVA: $F(2, 27) = 346.64$, $p < 0.001$, $\eta^2 = 0.963$). Group C (mixed organic waste; MOW) showed the highest reduction in recoverable residual substrate dry matter (83.54 ± 0.79%), followed by Group B (animal-derived food waste; AFW; 75.41 ± 1.99%) and Group A (vegetable and fruit waste; VFW; 63.23 ± 2.11%). Tukey's HSD test indicated significant pairwise differences among all three treatments ($p < 0.001$).

Importantly, although all experimental units received the same controlled cumulative fresh-substrate input (42.0 kg container^{-1} over 21 days) according to an identical feeding schedule, the treatments differed substantially in dry-matter content. Repeated measurements of fresh substrate dry matter at days 1, 7, 14, and 21 were therefore used together with the daily feeding records to estimate cumulative dry-matter input at the household-container level. The resulting cumulative dry-matter inputs were approximately 4.12 kg DM container^{-1} for VFW, 10.61 kg DM container^{-1} for AFW, and 8.20 kg DM container^{-1} for MOW.

At harvest, the entire recoverable residual substrate from each container was weighed separately on a fresh-mass basis, after which a representative sample from each container was dried to constant mass for determination of residual dry matter. Mean residual dry matter was 1.51 ± 0.09 kg in Group A, 2.60 ± 0.21 kg in Group B, and 1.35 ± 0.07 kg in Group C.

Because frass was separated from the residual substrate but was not quantified independently, the reported WR values represent the reduction in recoverable residual substrate dry matter rather than a complete dry-matter mass balance. Accordingly, differences among treatments should be interpreted as outcomes of the three practical household waste streams under fresh-mass-standardized feeding conditions and not as isolated effects of substrate composition or dry-matter supply.

Table 1. Reduction in recoverable residual substrate dry matter across three household organic waste-stream treatments after 21 days of BSFL rearing

Treatment	Estimated cumulative DM input (kg DM/container)	Residual substrate DM (kg/container)	WR (%)
Group A (Vegetable & Fruit Waste)	~4.12	1.51 ± 0.09	63.23 ± 2.11[a]
Group B (Animal-derived Food Waste)	~10.61	2.60 ± 0.21	75.41 ± 1.99[b]
Group C (Mixed Organic Waste)	~8.20	1.35 ± 0.07	83.54 ± 0.79[c]

Note: Values for residual substrate DM and WR are presented as mean ± SD (n = 10 independent household containers per treatment). All containers received 42.0 kg fresh substrate over 21 days according to the same controlled feeding schedule. Cumulative DM input was estimated using household-level substrate DM measurements obtained on days 1, 7, 14, and 21 together with the daily feeding records. Different superscript letters indicate significant differences according to Tukey's HSD test ($p < 0.001$). Frass was separated but not independently quantified; therefore, WR represents reduction in recoverable residual substrate DM rather than a complete system-level dry-matter mass balance.

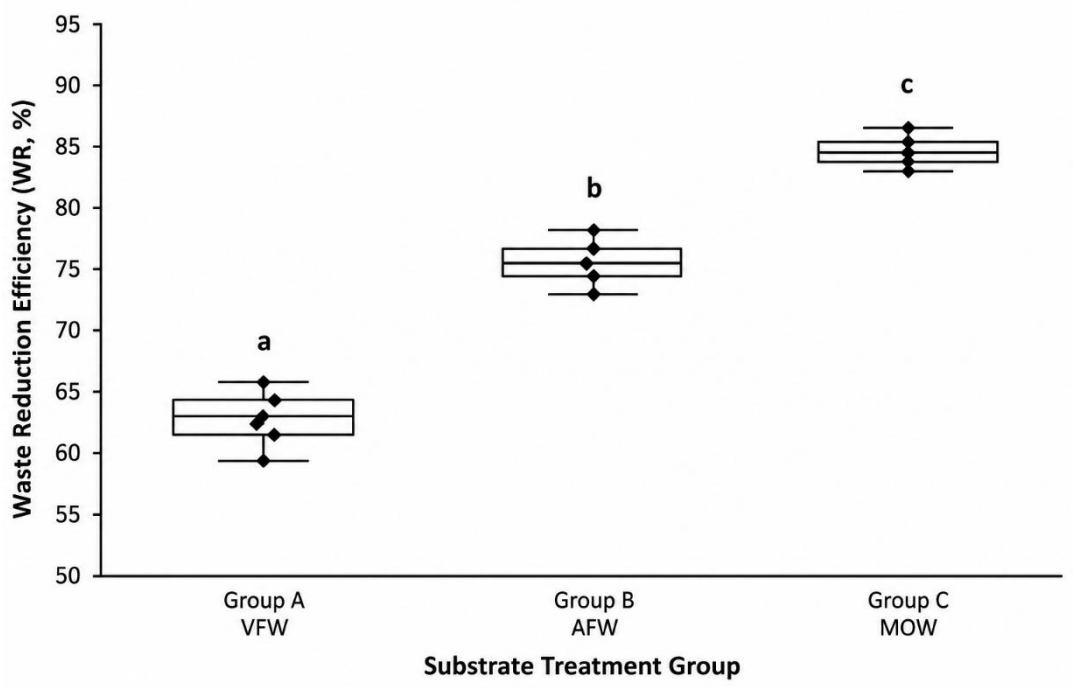

Figure 3. Distribution of reduction in recoverable residual substrate dry matter (WR, %) across the three household waste-stream treatments after 21 days of BSFL rearing (n = 10 independent household containers per treatment). Different letters indicate significant differences according to Tukey's HSD test ($p < 0.001$)

4.2. Larval Growth, Final Abundance, and Fresh Biomass

Larval growth and final fresh biomass differed markedly among the three household waste-stream treatments. At Day 21, Group B (AFW) produced the highest mean individual fresh mass (0.207 ± 0.004 g larva^{-1}) and directly measured total fresh larval biomass (439.6 ± 14.4 g container^{-1}), followed by Group C (MOW; 0.184 ± 0.004 g larva^{-1} and 396.0 ± 10.7 g container^{-1}) and Group A (VFW; 0.154 ± 0.004 g larva^{-1} and 325.2 ± 17.4 g container^{-1}). Total fresh larval biomass differed significantly among treatments ($F(2,27) = 160.65$, $p < 0.001$, $\eta^2 = 0.922$), with Tukey's HSD indicating significant pairwise differences among all three groups.

Mean larval body length showed the same treatment ranking at Day 21, with Group B exhibiting the greatest body length (2.902 ± 0.088 cm), followed by Group C (2.693 ± 0.096 cm) and Group A (2.378 ± 0.084 cm). The treatment effect on Day-21 body length was significant ($F(2,27) = 87.00$, $p < 0.001$, $\eta^2 = 0.866$), and all pairwise treatment comparisons were significant according to Tukey's HSD test.

Longitudinal measurements further showed that larval body length increased progressively from Day 7 to Day 21 in all three treatments (Figure 4). Across the monitoring period, Group B consistently exhibited the greatest mean body length, Group C showed intermediate values, and Group A showed the lowest values. Analysis accounting for repeated measurements at the household-container level indicated significant effects of treatment, rearing time, and their treatment × time interaction, demonstrating that larval growth trajectories differed among the three waste-stream treatments.

Final larval abundance was estimated for each container by dividing the directly measured total fresh larval biomass by the mean individual fresh mass obtained from a random subsample of 100 larvae from the same container. Estimated final abundance averaged approximately 2,107 larvae container^{-1} in Group A, 2,119 in Group B, and 2,153 in Group C. Because initial post-hatch larval abundance was not directly quantified, these estimates were not interpreted as survival rates.

Table 2. Day-21 larval growth, directly measured fresh biomass, and estimated final abundance across three household waste-stream treatments

Treatment	Mean body length (cm)	Mean individual fresh mass (g/larva)	Total fresh larval biomass (g/container)	Estimated final abundance (larvae/container)
Group A – VFW	2.373 ± 0.027[a]	0.154 ± 0.004[a]	325.2 ± 17.4[a]	2,107 ± 71
Group B – AFW	2.921 ± 0.038[b]	0.207 ± 0.004[b]	439.6 ± 14.4[b]	2,119 ± 30
Group C – MOW	2.730 ± 0.026[c]	0.184 ± 0.004[c]	396.0 ± 10.7[c]	2,153 ± 25

Note: Values are mean ± SD (n = 10 independent household containers per treatment). Total fresh larval biomass was measured directly from the complete larval harvest of each container after cleaning and standardized removal of surface moisture. Mean individual fresh mass was determined from a random subsample of 100 larvae from each container. Final larval abundance was subsequently estimated as total fresh larval biomass divided by mean individual fresh mass. Different superscript letters indicate significant differences among treatments according to Tukey's HSD test ($p < 0.05$). Final abundance is presented as a derived secondary outcome and was not used to infer survival because post-hatch initial larval abundance was not directly quantified.

Group B (Animal-derived Food Waste) showed the highest directly measured total fresh larval biomass (439.6 ± 14.4 g/container), followed by Group C (396.0 ± 10.7 g/container) and Group A (325.2 ± 17.4 g/container). A similar pattern was observed for mean larval body length at Day 21, with Group B showing the highest value (2.90 ± 0.09 cm), followed by Group C (2.69 ± 0.10 cm) and Group A (2.38 ± 0.08 cm). Significant differences were observed among all three treatments ($p < 0.001$; Table 2).

Mean larval body length increased from Day 7 to Day 21 in all treatments (Figure 4), with Group B consistently showing the highest values, followed by Groups C and A. Repeated-measures analysis further indicated significant effects of treatment, time, and their interaction ($p < 0.001$).

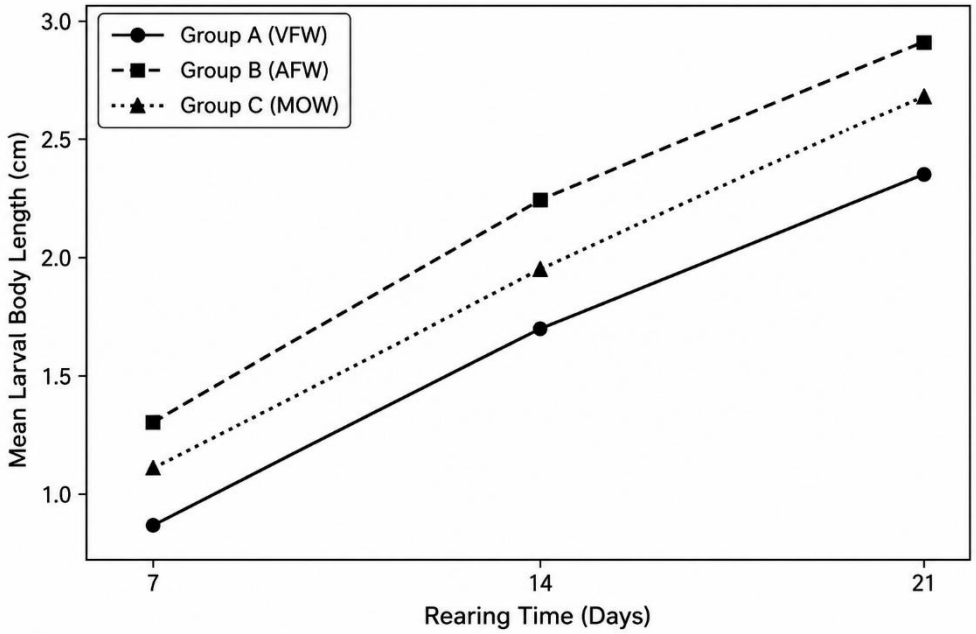

Figure 4. Changes in mean larval body length from Day 7 to Day 21 across the three substrate treatments. Values represent treatment means calculated from 10 independent household containers per treatment at each sampling time point.

4.3. NaOH-extractable Soluble Protein Content

The NaOH-extractable soluble protein content of BSFL biomass differed significantly among the three substrate treatments after 21 days of rearing ($p < 0.001$). Group B (Animal-derived Food Waste) showed the highest value (47.79 ± 1.04% DM), followed by Group C (Mixed Organic Waste; 44.56 ± 0.95% DM) and Group A (Vegetable and Fruit Waste; 39.95 ± 1.03% DM). Tukey's HSD test indicated significant differences among all three treatments ($p < 0.001$; Table 3)

Table 3. NaOH-extractable soluble protein content of black soldier fly larvae determined by the Bradford assay after 21 days of rearing

Treatment	NaOH-extractable soluble protein (% DM)
Group A (Vegetable & Fruit Waste)	$39{,}95 \pm 1{,}03^{a}$
Group B (Animal-derived Food Waste)	$47{,}79 \pm 1{,}04^{b}$
Group C (Mixed Organic Waste)	$44{,}56 \pm 0{,}95^{c}$

Note: Values are presented as mean ± SD, with n = 10 independent household-container biological replicates per treatment. Larval samples from each container were analyzed independently without pooling. Different superscript letters indicate significant differences among treatments according to Tukey's HSD test ($p < 0.05$).

B. DISCUSSION

4.4. **Effect of Substrate Type on Waste Reduction Efficiency**

Substrate type was associated with significant differences in waste reduction efficiency. Group C (Mixed Household Organic Waste) achieved the highest WR (83.54 ± 0.79%), followed by Group B (Animal-derived Food Waste; 75.41 ± 1.99%) and Group A (Vegetable and Fruit Waste; 63.23 ± 2.11%). This pattern is consistent with previous studies showing that substrate characteristics and composition can influence waste reduction performance in BSFL bioconversion systems (Meneguz et al. 2018; Bava et al. 2019; Lalander et al. 2019; Plaipintragarn et al. 2021; Amrul et al. 2022).

The higher WR observed in Group C may be related to substrate heterogeneity and differences in the availability and degradability of organic components, whereas vegetable- and fruit-based substrates may contain larger proportions of structural plant materials that are less readily degraded (Tucker et al. 2017; Gold et al. 2018). However, although all treatments received the same cumulative fresh-substrate input, their dry-matter inputs differed substantially. Therefore, the observed differences in WR cannot be attributed solely to substrate composition. Furthermore, because the chemical and microbial compositions of the substrates were not directly characterized, these mechanisms should be regarded as plausible explanations rather than direct experimental evidence. Within these limitations, Group C demonstrated the greatest reduction in recoverable residual substrate dry matter under the household-scale conditions evaluated in this study.

4.5. Larval Performance and Fresh Biomass

Substrate type was associated with clear differences in larval performance. After 21 days, Group B (Animal-derived Food Waste) showed the highest **directly measured total fresh larval biomass** and mean larval body length, followed by Groups C and A. This pattern is consistent with previous studies showing that substrate composition and nutrient availability can influence BSFL development and biomass production (Barragán-Fonseca et al. 2018; Meneguz et al. 2018; Nyakeri et al. 2017; Taufek et al. 2024).

The higher fresh larval biomass and body length observed in Group B may reflect differences in substrate characteristics and nutrient availability, as BSFL have been shown to exhibit physiological and digestive responses to different food substrates (Bonelli et al. 2020). Previous studies have also demonstrated that dietary nutrient concentration and substrate composition can influence larval development and biomass production (Barragán-Fonseca et al. 2018; Spranghers et al. 2017). However, although all treatments received the same cumulative fresh-substrate input, their dry-matter inputs differed substantially. Therefore, the higher larval biomass and body length observed in Group B cannot be attributed solely to substrate composition or nutrient quality. Moreover, because substrate chemical composition was not directly characterized, the mechanisms underlying these differences cannot be determined. The present findings therefore demonstrate differences in larval performance among the three practical household waste streams under fresh-mass-standardized feeding conditions, rather than establishing an independent nutrient-mediated effect of substrate type.

4.6. NaOH-Extractable Soluble Protein Content of Larval Biomass

The NaOH-extractable soluble protein content of BSFL biomass differed significantly among the substrate treatments, with the highest value observed in Group B, followed by Groups C and A. Previous studies have shown that rearing substrate composition can influence the nutritional composition of BSFL biomass, including protein-related characteristics (Spranghers et al. 2017; Chia et al. 2020; Pliantiangtam et al. 2021; Hopkins et al. 2021; Taufek et al. 2024). The present findings are therefore consistent with broader evidence that substrate characteristics are associated with differences in the nutritional composition of harvested BSFL biomass.

Importantly, protein content in the present study was determined using the Bradford assay following extraction with 0.1 M NaOH. Therefore, the reported values represent NaOH-extractable soluble protein rather than conventional crude protein determined from total nitrogen using methods such as Kjeldahl or Dumas (Bradford 1976). Consequently, the absolute values obtained in this study should not be directly compared with crude protein values reported using total-nitrogen methods. Instead, they should primarily be interpreted as relative differences among treatments analyzed using the same extraction and analytical procedure.

The higher NaOH-extractable soluble protein content observed in Group B may reflect differences in substrate characteristics and nutrient availability, as dietary composition has been reported to influence the nutritional composition of BSFL (Barragán-Fonseca et al. 2018; Spranghers et al. 2017). However, because dry-matter input differed among treatments despite the same cumulative fresh-substrate input, the observed differences cannot be attributed solely to substrate composition or nutrient quality. Furthermore, because the chemical composition of the experimental substrates and larval protein metabolism were not directly investigated, the mechanism underlying these differences cannot be determined. Therefore, the present results support an association between the three household waste-stream treatments and NaOH-extractable soluble protein content rather than establishing a direct causal relationship. Future studies should combine soluble-protein assessment with standardized total-protein determination and detailed substrate characterization to clarify this relationship.

4.7. Strengths, Limitations and Future Perspectives

A major strength of this study is its implementation under household-scale field conditions, which more closely reflects decentralized organic waste management practices than strictly controlled laboratory experiments. In addition, the simultaneous assessment of waste reduction, larval growth, directly measured total fresh larval biomass, and NaOH-extractable soluble protein content enabled the BSFL system to be evaluated from multiple perspectives under the same experimental conditions.

Nevertheless, several limitations should be acknowledged. First, although waste reduction efficiency (WR) was calculated on a dry-matter basis, substrate feeding was standardized at 42 kg fresh mass per container, resulting in unequal cumulative dry-matter inputs among treatments. Consequently, treatment effects cannot be attributed solely to substrate composition or nutrient quality. Second, the detailed chemical and microbial composition of the substrates was not characterized; therefore, direct relationships between specific substrate characteristics and larval performance could not be established. Third, protein was quantified using the Bradford assay following NaOH extraction and therefore represents NaOH-extractable soluble protein rather than conventional crude protein determined from total nitrogen using methods such as Kjeldahl or Dumas (Bradford 1976).

Fourth, initial stocking was based on an estimated number of eggs, and hatching success was not independently quantified. Although total fresh larval biomass at Day 21 was measured directly by weighing the complete harvested larval population from each container, final larval abundance was estimated from total fresh biomass and mean individual fresh mass. Therefore, final abundance should not be interpreted as an absolute survival estimate. Fifth, frass was separated but not independently quantified, and no larva-free control was included. Accordingly, the reported WR represents reduction in recoverable residual substrate dry matter rather than a complete system-level dry-matter mass balance, and the relative contributions of larval activity and other decomposition processes cannot be fully distinguished. Sixth, microbiological safety, heavy metals, and other potential contaminants in the larval biomass were not assessed. Finally, the experiment was conducted in a single geographical area and during a defined study period, which may limit the generalizability of the findings to other climatic and geographical conditions.

Future studies should standardize substrate loading on a dry-matter basis and directly quantify hatching success and final larval abundance to distinguish individual growth from population-level effects. Complete mass-balance assessments incorporating residual substrate, frass, larval biomass, and appropriate larva-free controls would further clarify the contribution of BSFL to organic-matter reduction. Detailed characterization of substrate composition and comprehensive nutritional analysis of BSFL biomass using standardized analytical methods are also recommended. Further studies should assess microbiological safety and potential contaminant accumulation, particularly when harvested larvae are considered for feed-related applications (Diener et al. 2015; Lopes et al. 2020). Multi-season and multi-location field trials would help determine the robustness of household-scale BSFL systems under different environmental conditions. In addition, economic and life-cycle assessments could provide a more comprehensive evaluation of the practical and environmental sustainability of decentralized BSFL bioconversion systems (Mertenat et al. 2019; Guo et al. 2021).

5. CONCLUSION

This household-scale field study demonstrated distinct performance patterns among three practical organic waste-stream treatments used for BSFL rearing in Ninh Binh, Vietnam. Under the fresh-mass-standardized feeding conditions applied in this study, mixed household organic waste achieved the highest waste reduction efficiency, whereas animal-derived food waste produced the highest directly measured total fresh larval biomass, mean larval body length, and NaOH-extractable soluble protein content. These findings demonstrate the potential of household-scale BSFL systems for decentralized organic waste reduction and resource recovery under local field conditions.

However, treatment differences should be interpreted with caution because substrate loading was standardized on a fresh-mass rather than dry-matter basis, resulting in unequal dry-matter inputs among treatments. In addition, hatching success was not quantified, substrate chemical composition was not characterized, and no larva-free control was included. Therefore, the observed differences should be interpreted as household-system outcomes rather than direct causal effects of substrate composition. Further multi-season and multi-location studies using standardized dry-matter loading and more complete mass-balance assessments are warranted.

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