

Feeding Frequency and Amino Acid Effects on Black Soldier Fly Larvae Performance

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Abstract

The continuous increase in waste generation, driven by population growth, necessitates sustainable waste management solutions. One promising approach is the bioconversion of organic waste using Black Soldier Fly (BSF) larvae, which naturally accelerate the decomposition process. This study investigates the effect of amino acid supplementation and feeding frequency on BSF larval growth, nutrient composition (protein, fat, carbohydrate, moisture, ash), waste reduction, and cultivation efficiency. A total of 700 five-day-old larvae were fed 1.8 g/larva/day using a mixture of restaurant and market waste. Results showed that the addition of 15 mL amino acids did not significantly enhance larval growth, except in fresh waste with a 70:30 ratio over three days, where larvae reached 27 g at 17 days old. Amino acid addition contributed to increased protein content. Feeding frequency impacted the Waste Reduction Index (WRI), waste reduction rate, and C/N ratio of residue. The highest WRI (5.27%) and reduction rate (94.90%) were observed in fresh P100 (1-day feeding), with residue C/N ratios ranging from 13.09 to 28.82. These findings highlight the potential of optimizing feed composition and frequency for enhanced BSF-based organic waste bioconversion.

Keywords: *amino acid, black soldier fly, feeding frequency, organic waste, residue*

Abstrak

Peningkatan terus-menerus dalam produksi sampah, didorong oleh pertumbuhan populasi, memerlukan solusi pengelolaan sampah yang berkelanjutan. Salah satu pendekatan yang menjanjikan adalah biokonversi sampah organik menggunakan larva Lalat Tentara Hitam (BSF), yang secara alami mempercepat proses dekomposisi. Studi ini menyelidiki efek suplementasi asam amino dan frekuensi pemberian pakan pada pertumbuhan larva BSF, komposisi nutrisi (protein, lemak, karbohidrat, kadar air, abu), pengurangan sampah, dan efisiensi budidaya. Sebanyak 700 larva berumur lima hari diberi makan 1,8 g/larva/hari menggunakan campuran sampah restoran dan pasar. Hasil penelitian menunjukkan bahwa penambahan 15 mL asam amino tidak meningkatkan pertumbuhan larva secara signifikan, kecuali pada sampah segar dengan rasio 70:30 selama tiga hari, di mana larva mencapai 27 g pada umur 17 hari. Penambahan asam amino berkontribusi pada peningkatan kandungan protein. Frekuensi pemberian pakan memengaruhi Indeks Pengurangan Sampah (WRI), tingkat pengurangan sampah, dan rasio C/N residu. WRI tertinggi (5,27%) dan tingkat pengurangan (94,90%) diamati pada P100 segar (pemberian pakan 1 hari), dengan rasio C/N residu berkisar antara 13,09 hingga 28,82. Temuan ini menyoroti potensi mengoptimalkan komposisi dan frekuensi pakan untuk meningkatkan biokonversi sampah organik berbasis BSF.

Kata Kunci: *asam amino, lalat tentara hitam, frekuensi pemberian makan, sampah organik, residu*

1. Introduction

The generation of solid waste continues to escalate annually, in parallel with rapid population growth, urbanization, and shifts in consumption behavior. This growing volume of waste presents increasing challenges for waste management, particularly in developing countries [1]. Waste is defined as the residual material from human activities that no longer hold value, typically in solid or semi-solid form [2]. According to the National Waste Management Information System [3], food waste represents the largest proportion of total waste in Indonesia, accounting for 39.17%. The Food Sustainability Index (2021) further reports that Indonesia generates approximately 121 kg of food waste per capita annually, with an estimated 63% originating from household sources.

One promising strategy for addressing organic waste, including food waste, is bioconversion using Black Soldier Fly (BSF) larvae (*Hermetia illucens*). BSF larvae are known for their efficiency in degrading organic waste through their gut microbiota, which aids in the decomposition of complex organic matter [4].

The BSF life cycle spans approximately 41 days, consisting of the larval, prepupal, pupal, and adult stages. BSF larvae can reduce organic waste mass by 52–56% [5], with some reports indicating weight reduction of up to 80% [6]. In addition to effective waste reduction, BSF larvae offer high nutritional value, containing 41–42% crude protein, 31–35% fat (ether extract), 14–15% ash, 4.8–5.1% calcium, and 0.6–0.63% phosphorus in dry weight [7].

The end-products of BSF bioconversion have considerable commercial potential. The larvae can serve as an alternative protein source for aquaculture and poultry industries due to their high protein and fat content. At the same time, the leftover byproduct from the larvae, known as frass or residue or *kasgot*, consists of a mixture of larval excrement, uneaten substrate (the organic material or waste that serves as a food source for BSF larvae), and shed exoskeletons. This material is rich in nutrients and commonly used as an organic fertilizer. Frass contains essential macro- and micronutrients required for plant growth, including 3.28% nitrogen, 3.39% phosphorus, 9.74% potassium, 40.95% organic carbon, a C/N (carbon-to-nitrogen) ratio of 12.50, and 11.04% moisture content [4]. The nutrient profile of frass meets the technical criteria set by Indonesia's Ministry of Agriculture Regulation No. 261 of 2019, and its C/N ratio aligns with compost standards as per SNI 19-7030-2004 [8].

Among the primary sources of organic waste in Indonesia are markets and restaurants. Market waste is predominantly organic and can be valorized through proper segregation and application of the 3R principles (Reduce, Reuse, and Recycle) as mandated in Law No. 18 of 2008 [9], [10]. Meanwhile, restaurants generate large quantities of food-related waste, with organic matter comprising approximately 76.66% of total restaurant waste, including food scraps (38.23%), spoiled foodstuffs (14.60%), and vegetable residues (8.24%) [11].

To optimize BSF cultivation, nutrient-rich feed is essential. The addition of amino acids, as the fundamental building blocks of proteins, may enhance larval development and improve biomass quality. Essential amino acids such as tryptophan, lysine, and threonine serve specific biological roles: tryptophan regulates appetite hormones, lysine contributes to tissue and enzyme formation, and threonine supports immune function. Supplementing BSF feed with these amino acids may improve growth performance and nutritional composition of the larvae.

This study aims to investigate the effects of feeding frequency and amino acid supplementation on the growth performance, nutrient content, and bioconversion efficiency of BSF larvae reared on a mixture of market and restaurant waste. The findings are expected to contribute to the development of more sustainable organic waste management systems and enhanced BSF-based biomass production.

2. Material and Methods

Preliminary Research

Preliminary trials included the determination of feed types based on amino acid supplementation levels. Two concentrations were selected: 0 mL (control) and 15 mL, based on prior studies involving amino acid addition in BSF-based chicken feed formulations [12]. Amino acids are the fundamental components of proteins and play a key role in larval growth and metabolic function [13]. This study aims to examine the novel application of amino acids in mixed organic waste substrates and assess their effects on BSF larval growth and biomass productivity. The growth of larvae was assessed by measuring the weight of a representative sample, comprising 10% of the larval population, at regular three-day intervals. The measurements began when the larvae were five days old, and continued until day 23, resulting in growth data for days 5, 8, 11, 14, 17, 20, and 23. This method allowed for systematic tracking of larval development over time, providing insights into growth rates and identifying peak biomass periods. The Waste Reduction Index (WRI) is commonly used to evaluate the efficiency of organic waste reduction during the bioconversion process. It measures how effectively BSF larvae convert waste into biomass over time. The waste reduction rate is determined by comparing the initial amount of waste with the remaining non-degraded residue in amount of time. A higher WRI indicates a more efficient bioconversion process. The calculation of WRI is presented in Equation 1 [14].

$$WRI (\%) = \frac{\text{total substrate (g)} - \text{residue (g)}}{\text{total substrate (g)} \times \text{days of trial (d)}} \quad (1)$$

The waste used in preliminary research was fresh, sourced daily to ensure consistency and minimize degradation. Based on the experimental variables, two reactors were prepared for preliminary testing. The main study later employed 24 reactors, each with two replicates per treatment group. The variation of preliminary research can be seen in **Table 1**. The waste sources are restaurant (R) and market (M). The

BSF larvae used were five days old, selected for their active feeding stage and adaptability to organic substrates. At this age, each larva weighs approximately 2.26 mg. Considering the reactor dimensions (64 x 47 cm) and feeding design (1,8 g/larvae.day), 700 larvae (approximately 1.5 g total weight) were used per reactor to ensure uniform larval density and optimal substrate contact. The flow diagram of the preliminary research experiment can be seen in **Fig. 1**.

Table 1. Preliminary Research

| Waste source | Amino acid (mL) | Feeding frequency |
|--------------|-----------------|-------------------|
| R:M (50:50) | 0 | Once a day |
| R:M (50:50) | 15 | Once a day |

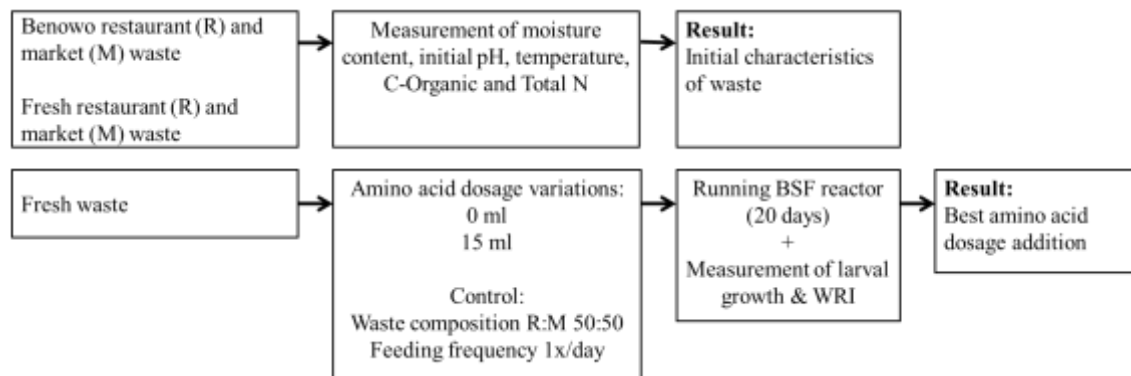


Fig. 1: Flow diagram of preliminary research

Main Research

The main research aimed to assess the impact of feeding frequency and amino acid supplementation on the growth performance, nutritional content, and waste reduction efficiency of BSF larvae. Two types of organic waste were utilized as feed: Benowo landfill waste, representing a mixed post-consumer waste stream, and fresh waste, which consisted of newly collected restaurant and market waste and served as the control substrate. Four different waste composition treatments were tested to reflect variations in organic matter: 100% restaurant waste (R100), 100% market waste (M100), restaurant to market waste in a 50:50 ratio, and restaurant to market waste in a 70:30 ratio.

In addition to substrate composition, two feeding frequency regimes were applied: feeding once per day and feeding once every three days. Each treatment was tested under two amino acid supplementation levels: 0 mL (control) and 15 mL. The amino acid mix used in this study included three essential amino acids (tryptophan, lysine, and threonine) selected for their physiological roles in enhancing protein synthesis, tissue development, and immunity in larvae.

Measurements conducted during the experiment included organic waste reduction, larval growth, larval nutritional content, and frass analysis. Organic waste reduction was evaluated using the WRI and the percentage of substrate mass reduction. Larval growth was monitored by measuring larval weight and body length periodically throughout the cultivation period. To assess the nutritional value of the harvested larvae, analyses were performed to determine their crude protein, fat, carbohydrate (by difference), moisture content, and total ash levels.

The proximate analysis of the samples was conducted using standardized methods. Moisture content was determined by the gravimetric method in accordance with SNI 01-2891-1992 (point 5.1), by drying a known mass of sample in an oven until a constant weight was reached. Total ash content was measured using the gravimetric method following SNI 01-2891-1992 (point 6.1), involving incineration of the sample in a muffle furnace to obtain inorganic residue. Protein was analyzed using the Kjeldahl method with a Kjeltex apparatus, based on SNI 01-2891-1992 (point 7.1), which involved digestion, distillation, and titration to determine nitrogen content and subsequent conversion to protein. Total fat content was determined using the Soxhlet-hydrolysis method according to IKP/K-1, involving acid hydrolysis followed by solvent extraction. Carbohydrate content was calculated by difference using the method outlined in IKP/K-3, by subtracting the total percentages of moisture, ash, protein, and fat from 100%.

Frass, the residue produced by the larvae during the bioconversion process, was also analyzed. Key parameters measured in frass included moisture content, pH, temperature, and carbon-to-nitrogen (C/N) ratio. These indicators help determine the potential of frass as organic fertilizer. The organic carbon (C) content was measured using a gravimetric method [15]. Nitrogen content was determined through the

analysis of Total Kjeldahl Nitrogen (TKN), following the EPA-NERL Method 351.3, which quantifies both organic nitrogen and ammonia. All experimental treatments were carried out in duplicate, using a total of 24 reactors. Each reactor was filled with 700 five-day-old larvae, as established in the preliminary study, to maintain consistency in larval density across treatments. The flow diagram of the main research experiment can be seen in **Fig. 2**. This main study provides comprehensive data on how feeding frequency and amino acid supplementation affect BSF larval productivity and the valorization of organic waste through bioconversion.

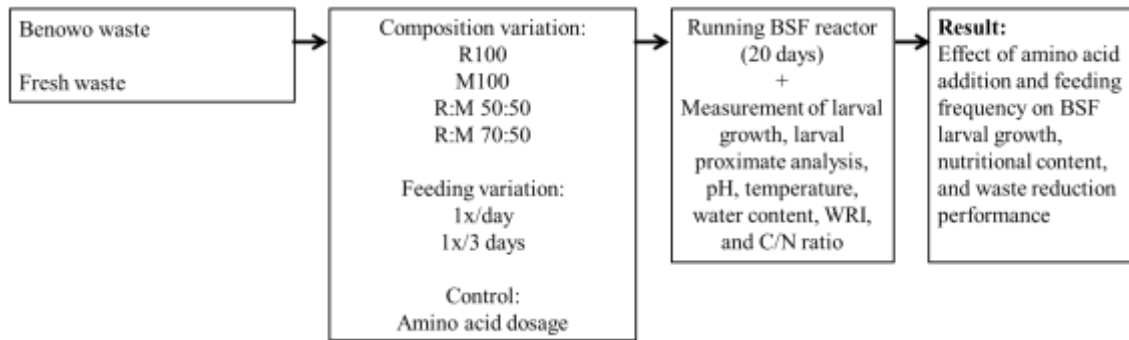


Fig. 2: Flow diagram of main research

3. Results and Discussion

This research discusses the effects of feeding frequency and amino acid supplementation on the performance of BSF larvae, including growth, nutrient composition, and waste reduction efficiency.

Preliminary Research

Preliminary research was conducted to obtain essential baseline data to guide the design of the main experiment. This stage focused on evaluating the impact of amino acid supplementation at two levels (0 mL and 15 mL) on the growth performance of BSF larvae and their waste bioconversion efficiency. Key parameters measured included larval weight, and the amount of residue or frass produced. Larval growth can be seen in **Fig. 3**, meanwhile WRI and frass produced can be seen in **Table 2**.

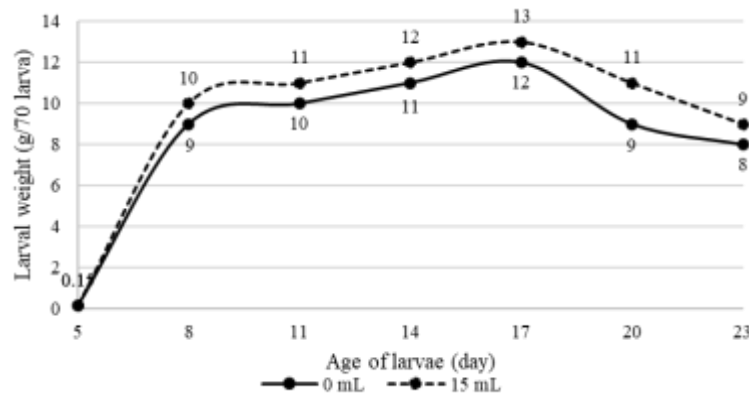


Fig. 3: Larval growth in preliminary research

Table 2. Frass and waste reduction index (WRI) of preliminary research

| Amino acid (mL) | Initial weight (kg) | Frass weight (kg) | WRI (%) |
|-----------------|---------------------|-------------------|---------|
| 0 | 25.20 | 1.0068 | 4.79 |
| 15 | 25.20 | 1.005 | 4.80 |

Larvae supplemented with 15 mL of amino acids showed greater biomass gain and faster development compared to those without supplementation, although the differences in weight were not statistically significant. By day 21, more larvae in the 15 mL group had reached the prepupal stage and migrated to the designated medium, indicating accelerated growth. Substrate with higher protein content can reduce developmental time, amino acids are the building of proteins [16]. Waste reduction was also evaluated using the WRI, which measures the efficiency of BSF larvae in degrading organic material over a 20-day period. The highest WRI values were observed in the 15 mL treatment, reflecting enhanced waste

reduction performance. The higher the WRI, the greater the ability of BSF larvae to reduce organic matter [17]. Based on improved larval development and superior WRI results, the 15 mL amino acid concentration was selected for the main experimental design.

Larval Growth

The average larval weight has an upward trend until the age of 17 days and decreases thereafter. The larval growth data can be seen in Table 3. BSF larvae are known for their rapid growth and ability to develop significantly within a short cultivation period. Periodic weight measurements were essential to obtain consistent data on larval biomass accumulation under different treatment conditions and to assess the effects of feed composition and feeding frequency on larval development. The results showed that the addition of 15 mL of amino acids did not lead to a statistically significant increase in larval weight compared to the control. However, the highest larval growth was observed in the treatment using Benowo landfill waste with a 50:50 ratio of restaurant to market waste and a feeding frequency of once every three days, where biomass peaked at 23–27 g for 70 larvae. This suggests that waste composition and feeding frequency have a more substantial impact on larval growth than amino acid supplementation alone.

These findings emphasize the importance of optimizing substrate quality and feeding strategies to enhance BSF larval growth, which is crucial for improving biomass yield and productivity in large-scale cultivation. Different types of amino acid such as methionine can improve larval growth [18]. At 20 days of age, all treatment groups showed a decrease in larval weight. This occurred because the larvae had entered the prepupal stage. During this phase, larvae become less active in consuming organic waste, their movement slows down, and their bodies begin to shrink as hardened pupal skin forms. These changes lead to a reduction in larval weight [8].

Table 3. Larval growth of main research

| Source | Variations | Larval weight (g/70 larva) | | | | | | |
|--------------------|---------------|----------------------------|--------|---------|---------|---------|---------|---------|
| | | 5 days | 8 days | 11 days | 14 days | 17 days | 20 days | 23 days |
| Benowo | R100 (1 day) | 0.15 | 3 | 16 | 20 | 23 | 16 | 15 |
| | M100 (1 day) | 0.15 | 3 | 15 | 20 | 24 | 16 | 14 |
| | 50:50 (1 day) | 0.15 | 4 | 13 | 22 | 24 | 15 | 12 |
| | 70:30 (1 day) | 0.15 | 3 | 14 | 22 | 25 | 16 | 14 |
| | R100 (3 day) | 0.15 | 4 | 10 | 20 | 22 | 18 | 11 |
| | M100 (3 day) | 0.15 | 3 | 9 | 13 | 15 | 13 | 10 |
| | 50:50 (3 day) | 0.15 | 3 | 10 | 16 | 25 | 15 | 13 |
| | 70:30 (3 day) | 0.15 | 3 | 19 | 23 | 27 | 21 | 13 |
| Fresh (control) | R100 (1 day) | 0.15 | 3 | 11 | 21 | 22 | 20 | 14 |
| | M100 (1 day) | 0.15 | 3 | 6 | 12 | 15 | 12 | 10 |
| | 50:50 (1 day) | 0.15 | 4 | 12 | 21 | 22 | 15 | 13 |
| | 70:30 (1 day) | 0.15 | 4 | 14 | 21 | 22 | 18 | 15 |
| | R100 (3 day) | 0.15 | 4 | 8 | 20 | 21 | 17 | 16 |
| | M100 (3 day) | 0.15 | 4 | 12 | 20 | 24 | 15 | 12 |
| | 50:50 (3 day) | 0.15 | 3 | 6 | 19 | 20 | 15 | 13 |
| | 70:30 (3 day) | 0.15 | 3 | 10 | 15 | 21 | 17 | 13 |

Larval content

To evaluate the nutritional composition of larvae, proximate analysis was conducted on selected samples at 18 days of age. This timing aligns with the larvae's transition into the prepupal phase, which typically occurs at instar 6 and is generally reached around 18 days of development [3]. At this stage, larvae have accumulated significant biomass, making them suitable for nutritional evaluation. Due to limitations in sample processing, only selected treatment groups were analyzed. The selection was based on larval biomass performance, which is indicative of optimal larval development. The results of the proximate analysis are presented in Table 4.

Table 4. Results of larval proximate analysis of main research

| Source | Variations | Moisture content (%) | Total ash (%) | Protein (%) | Fat (%) | Carbohydrate (%) |
|--------|--------------|----------------------|---------------|-------------|---------|------------------|
| Benowo | R100 (1 day) | 61 | 21.94 | 39.00 | 39.00 | 0.14 |
| | M100 (1 day) | 65 | 18.90 | 40.47 | 40.47 | 0.14 |

Table 4. Results of larval proximate analysis of main research

| Source | Variations | Moisture content (%) | Total ash (%) | Protein (%) | Fat (%) | Carbohydrate (%) |
|-----------------|-----------------|----------------------|---------------|-------------|---------|------------------|
| Fresh (control) | R70:M30 (3 day) | 63 | 18.67 | 36.08 | 45.11 | 0.15 |
| | R100 (1 day) | 63 | 26.29 | 33.81 | 39.78 | 0.14 |
| | M100 (1 day) | 72 | 25.88 | 34.00 | 40.00 | 0.12 |
| | R70:M30 (3 day) | 62 | 21.54 | 46.97 | 31.31 | 0.12 |

The highest moisture content observed was 72% in the M100 (1-day) fresh waste treatment, while the highest total ash content was 26.29% in the R100 (1-day) treatment, and the highest protein content reached 46.97% in the 70:30 (3-day) fresh waste treatment. These three values were all obtained from treatments using fresh waste. In contrast, the highest fat and carbohydrate contents (45.11% and 0.15%, respectively) were found in the 70:30 (3-day) treatment using Benowo landfill waste. Protein showed the highest percentage, which can be attributed to the addition of 15 mL of amino acids in the feed. This supports the conclusion that amino acid supplementation can increase protein levels in BSF larvae, as amino acids are the primary building blocks of protein [13]. Protein content in BSF larvae typically ranges from 40–55%, while fat content ranges from 29–32% [19], [20], [21]. The ash content in this study was also relatively high, ranging from 18.67% to 26.29%, exceeding the typical range of 7.5–10.6% [22]. Ash content reflects the mineral composition of BSF larvae after all organic matter has been incinerated at high temperatures. Notably, ash content may be influenced by the development of the cuticle layer as the larvae enter the prepupal stage [23].

Temperature and pH

Initial pH measurements were essential for determining baseline conditions and evaluating their suitability for BSF larval growth. These values ranged from 6.9 to 7.0. In addition to pH, the substrate temperature was monitored to ensure conditions were within the suitable range for BSF development. The temperatures ranged from 31.2 to 33.5°C. Temperature and pH can be seen in **Table 5**.

Table 5. Temperature and pH of main research

| Source | Variations | Initial | | After amino acid addition | | Frass | |
|-----------------|---------------|---------|-------------|---------------------------|-------------|-------|-------------|
| | | pH | Temperature | pH | Temperature | pH | Temperature |
| Benowo | R100 (1 day) | 7 | 31.3 | 6.7 | 32.3 | 7 | 31.25 |
| | M100 (1 day) | 6.9 | 31.5 | 6.7 | 31.6 | 7 | 32.45 |
| | 50:50 (1 day) | 7 | 32.2 | 7 | 31.6 | 7 | 33.15 |
| | 70:30 (1 day) | 7 | 33.1 | 7 | 33.4 | 7 | 31.7 |
| | R100 (3 day) | 7 | 32.6 | 7 | 33.1 | 7 | 32.35 |
| | M100 (3 day) | 7 | 31.8 | 7 | 31.8 | 7 | 33.15 |
| | 50:50 (3 day) | 6.9 | 32.5 | 6.8 | 32.6 | 7 | 31.75 |
| | 70:30 (3 day) | 7 | 31.5 | 7 | 31.6 | 7 | 32.25 |
| Fresh (Control) | R100 (1 day) | 7 | 31.9 | 7 | 32.6 | 7 | 32.5 |
| | M100 (1 day) | 6.9 | 32.2 | 6.9 | 31.7 | 7 | 33.2 |
| | 50:50 (1 day) | 7 | 33.1 | 7 | 32.3 | 7 | 31.8 |
| | 70:30 (1 day) | 7 | 32.2 | 7 | 31.5 | 7 | 32.6 |
| | R100 (3 day) | 7 | 33.5 | 7 | 31.8 | 7 | 31.5 |
| | M100 (3 day) | 6.9 | 31.4 | 7 | 31.2 | 7 | 32.3 |
| | 50:50 (3 day) | 7 | 31.6 | 7 | 33.2 | 7 | 31.2 |
| | 70:30 (3 day) | 7 | 32.2 | 7 | 31.7 | 7 | 32.9 |

pH falls within the optimal range for BSF substrate pH, reported to be between 6.0 and 8.0 [24]. After amino acid addition, the pH values ranged from 6.7 to 7.0, indicating a slight decrease. Amino acids addition tends to decrease pH [25]. This decline is likely due to microbial activity breaking down organic matter into organic acids, a known byproduct of microbial decomposition processes in waste substrates [26]. In addition to pH, the substrate temperature was monitored to ensure conditions were within the suitable range for BSF development. Prior to amino acid addition, temperatures ranged from 31.3°C to 33.5°C, and after amino acid addition, from 31.2°C to 33.4°C. These temperature values fall within the optimal range for BSF larval growth, which lies between 30°C and 36°C. To prevent sudden temperature fluctuations, all experimental reactors were placed in shaded environments, away from direct sunlight that can increase the death rate [27]. Amino acid addition may also influence the chemical environment of the

waste, potentially altering water stability. A decrease in pH may promote the breakdown or evaporation of unstable water compounds, thereby reducing overall moisture content. This underscores the need to assess not only chemical but also physical parameters in evaluating substrate suitability.

Moisture content

In this study, moisture content was measured before and after amino acid addition to assessing the impact of amino acids on water availability in the substrate. Moisture content can be seen in **Table 6**.

Table 6. Moisture content of main research

| Source | Variations | Initial | After amino acid addition | Frass |
|--------------------|---------------|---------|---------------------------|-------|
| Benowo | R100 (1 day) | 78% | 43% | 38% |
| | M100 (1 day) | 95% | 93% | 38% |
| | 50:50 (1 day) | 77% | 59% | 38% |
| | 70:30 (1 day) | 79% | 70% | 39% |
| | R100 (3 day) | 82% | 46% | 39% |
| | M100 (3 day) | 95% | 92% | 38% |
| | 50:50 (3 day) | 74% | 70% | 39% |
| | 70:30 (3 day) | 84% | 65% | 38% |
| Fresh (control) | R100 (1 day) | 78% | 69% | 38% |
| | M100 (1 day) | 95% | 95% | 40% |
| | 50:50 (1 day) | 73% | 85% | 37% |
| | 70:30 (1 day) | 84% | 63% | 40% |
| | R100 (3 day) | 75% | 71% | 38% |
| | M100 (3 day) | 95% | 94% | 38% |
| | 50:50 (3 day) | 76% | 61% | 39% |
| | 70:30 (3 day) | 83% | 59% | 38% |

Moisture is a key factor influencing the substrate's texture and, subsequently, the larvae's ability to consume feed, move within the medium, and grow effectively [28]. The highest initial moisture content was observed in the 100% market waste variation, reaching 95%, likely due to the high proportion of water-rich fruit waste. In contrast, other waste compositions exhibited more moderate moisture levels. Variation in moisture content across samples is attributed to inconsistent waste mixing and the heterogeneous nature of the organic materials. Moisture content is a crucial determinant of BSF larval performance, as larvae thrive best within a moisture range of 65–90% [29]. A substrate that is sufficiently moist supports enzymatic activity in the larvae's gut and facilitates waste digestion [30]. Together, the data on pH, temperature, and moisture content indicate that most treatment conditions provided a favorable environment for BSF larvae. These parameters are essential for optimizing substrate conditions to enhance larval development and bioconversion efficiency.

WRI and organic waste reduction

In this study, the organic waste reduction achieved by BSF larvae ranged from 92.88% to 94.90%. WRI and reduction can be seen in **Table 7** **Error! Reference source not found.**

Table 7. WRI and organic waste reduction of main research

| Source | Variations | WRI (%) | Reduction (%) |
|--------------------|---------------|---------|---------------|
| Benowo | R100 (1 hari) | 5.21 | 93.73 |
| | R100 (1 day) | 5.22 | 93.95 |
| | M100 (1 day) | 5.22 | 93.93 |
| | 50:50 (1 day) | 5.23 | 94.06 |
| | 70:30 (1 day) | 5.21 | 93.84 |
| | R100 (3 day) | 5.23 | 94.19 |
| | M100 (3 day) | 5.24 | 94.23 |
| | 50:50 (3 day) | 5.23 | 94.13 |
| Fresh (control) | 70:30 (3 day) | 5.24 | 94.30 |
| | R100 (1 day) | 5.27 | 94.90 |
| | M100 (1 day) | 5.18 | 93.26 |
| | 50:50 (1 day) | 5.23 | 94.15 |
| | 70:30 (1 day) | 5.16 | 92.88 |
| | R100 (3 day) | 5.23 | 94.13 |

Table 7. WRI and organic waste reduction of main research

| Source | Variations | WRI (%) | Reduction (%) |
|--------|---------------|---------|---------------|
| | M100 (3 day) | 5.23 | 94.06 |
| | 50:50 (3 day) | 5.19 | 93.31 |

The highest reduction rate, 94.90%, was observed in the treatment that combined optimal substrate composition with effective feeding frequency and amino acid supplementation. This result demonstrates that the specific combination of feeding frequency and substrate composition can enhance larval performance and waste degradation. The organic waste reduction rate is directly proportional to the larval ability to break down organic matter. A higher reduction rate indicates a more efficient bioconversion process, which is critical in the context of sustainable waste management [31]. The WRI, which accounts for both the extent of waste reduction and the time taken to achieve it, serves as a dynamic performance metric to evaluate treatment efficiency. WRI values correspond with the organic reduction rates, affirming that larvae maintained high degradation efficiency across all treatment groups. These findings suggest that BSF larvae can achieve substantial organic matter reduction within a short cultivation period, reinforcing their potential as a biowaste treatment agent. The WRI value was higher compared to BSF reared on substrates containing kitchen waste, vegetable waste, and fruit waste [14], [32].

C/N ratio

The C/N ratio was measured before amino acid addition, after amino acid addition, and in the residual frass after bioconversion. This assessment was carried out to evaluate the impact of BSF larval activity on the decomposition process and nutrient transformation within the substrate. C/N ratio can be seen in Table 8. Microorganisms, such as bacteria and fungi, play a significant role in utilizing amino acids as nutrient sources for their metabolic activities. These microbes break down amino acids into simpler compounds, such as organic acids and carbon-rich molecules, contributing to fluctuations in C-Organic levels. Additionally, protein content in the substrate (partly derived from amino acid supplementation) increases nitrogen availability, which is further converted into ammonia by heterotrophic bacteria, thereby elevating TKN values [33]. The resulting C/N ratios in the process residues ranged from 13.09 to 28.82, indicating variation in substrate composition, microbial activity, and degradation efficiency. Restaurant waste generally exhibits a high C/N ratio (33.69), while market waste tends to have a more balanced ratio between 10 and 20, aligning with the suggested compost quality and standard in SNI 19-7030-2004 [9], [11], [34]. Residues with C/N ratios in this optimal range are suitable for composting.

Table 8. C/N ratio of main research

| Source | Variations | Initial | After amino acid addition | Frass |
|---------------|-----------------|--------------|---------------------------|-------|
| Benowo | R100 (1 day) | 11.89 | 17.30 | 23.72 |
| | M100 (1 day) | 15.02 | 25.96 | 15.70 |
| | 50:50 (1 day) | 17.03 | 10.91 | 28.82 |
| | 70:30 (1 day) | 13.49 | 10.28 | 18.51 |
| | R100 (3 day) | 28.63 | 17.34 | 19.27 |
| | M100 (3 day) | 7.75 | 25.25 | 14.97 |
| | 50:50 (3 day) | 8.77 | 19.81 | 18.30 |
| | 70:30 (3 day) | 19.94 | 11.27 | 22.39 |
| | Fresh (control) | R100 (1 day) | 15.51 | 10.58 |
| M100 (1 day) | | 8.17 | 35.17 | 17.31 |
| 50:50 (1 day) | | 3.33 | 12.41 | 20.24 |
| 70:30 (1 day) | | 14.78 | 31.47 | 17.03 |
| R100 (3 day) | | 7.61 | 26.91 | 13.09 |
| M100 (3 day) | | 7.96 | 26.39 | 16.20 |
| 50:50 (3 day) | | 5.90 | 20.32 | 14.16 |
| | 70:30 (3 day) | 23.20 | 7.93 | 16.38 |

In this study, several residue samples met the composting C/N standard, indicating potential for use as organic compost. According to SNI 19-7030-2004, other compost quality parameters include a moisture content of ≤50%, pH between 6.8–7.49, a minimum nitrogen content of 0.4%, and organic matter content ≥27%. While some of these criteria (such as pH, moisture, temperature, and nitrogen) were fulfilled, further compost maturation through mesophilic, thermophilic, and stabilization phases may be necessary for full compliance. Notably, some samples without amino acid supplementation already had C/N ratios within the

ideal range, suggesting that the natural composition of market and restaurant waste may suffice for composting. A significant finding in the mass balance analysis was observed in the fresh waste sample M100 (1-day feeding interval), which produced the lowest residue mass of 1.153 kg, indicating the highest bioconversion efficiency by BSF larvae, especially in market waste.

4. Conclusion

This study evaluated the effects of feeding frequency and amino acid supplementation on BSF larval growth, nutritional content, and waste reduction performance. The addition of 15 mL amino acids did not significantly impact larval growth, except in the fresh waste 70:30 (3-day feeding) treatment, where 17-day-old larvae reached 27 g per 70 larvae. Nutritional content was primarily influenced by waste composition. The highest protein content (46.97%) was found in fresh waste 70:30 (3 days), and the highest fat content (45.11%) in Benowo waste 70:30 (3 days). The feeding frequency had limited effect on nutrient composition but influenced waste reduction outcomes. The highest WRI (5.27%) and reduction rate (94.90%) were achieved with fresh market waste (M100, 1-day feeding). Residue C/N ratios ranged from 13.09 to 28.82, with several within the compostable range. In summary, waste composition and feeding strategy are more influential than amino acid addition in optimizing BSF cultivation for nutrient recovery and organic waste management.

In this research, the specific composition of materials in market waste and restaurant waste was not analyzed. The collected waste was only separated from non-biodegradable components such as plastic and metal. For future research, it is recommended to use pre-sorted waste to ensure a more homogeneous substrate, allowing for better control over the composition used for BSF feeding. Additionally, this study did not perform a proximate analysis of the substrate. Conducting such an analysis in future work would be beneficial to investigate the relationship between feeding frequency and amino acid supplementation with the changes in proximate composition between the substrate and the resulting larvae.

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6. Abbreviations

| | |
|-----------------|---|
| <i>BSF</i> | Black Soldier Fly |
| <i>WRI</i> | Waste Reduction Index |
| <i>SNI</i> | Indonesian National Standard |
| <i>C/N</i> | Carbon-to-nitrogen |
| <i>TKN</i> | Total Kjeldahl Nitrogen |
| <i>EPA-NERL</i> | Environmental Protection Agency–National Exposure Research Laboratory |

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