



Nutritional Potential of *Tenebrio molitor* Larvae Produced from Organic Waste Bioconversion as an Alternative Feed Ingredient: Evaluation Against Indonesian National Feed Standards for Broiler, Layer, and Fish Feedstock

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ABSTRACT

The increasing demand for sustainable protein sources and the accumulation of organic waste have encouraged the development of insect-mediated bioconversion for resource recovery. This study evaluated the nutritional quality of *Tenebrio molitor* larvae reared on various organic waste substrates and assessed their suitability as an alternative feed ingredient in accordance with the Indonesian National Standards (SNI). A laboratory-scale factorial experiment was conducted using three substrate treatments: household food waste (S1), a 1:1 mixture of household food waste and seafood waste (S2), and seafood waste (S3), combined with two larval densities (0.5 and 1 larva cm⁻²). Harvested larvae were analyzed for crude protein, crude fat, moisture, ash, and carbohydrate contents, and data were evaluated using two-way ANOVA. Substrate composition, larval density, and their interaction significantly affected all proximate parameters ($p < 0.001$). The highest crude protein content (37.16%) was obtained from larvae reared on seafood waste at 1 larva cm⁻² (S3-D1), while carbohydrate content decreased with increasing seafood waste proportions. Crude fat ranged from 24.80–27.14%, moisture from 10.10–12.25%, and ash from 9.60–10.73%. The larvae met SNI requirements for protein and moisture in broiler, layer, and tilapia feeds. These findings demonstrate that *T. molitor* effectively converts organic waste into protein-rich biomass, supporting its application as a sustainable feed ingredient and promoting circular resource management.

Contribution to Sustainable Development Goals (SDGs):

SDG 2: Zero Hunger

SDG 12: Responsible Consumption

SDG 13: Climate Action

SDG 15: Life on Land

1. INTRODUCTION

1.1. Research Background

The rapid growth of the global population, urbanization, and changing consumption patterns have substantially increased food waste generation worldwide. According to the United Nations

Environment Programme (UNEP), approximately 1.05 billion tonnes of food waste were generated globally in 2022, representing nearly 19% of the food available to consumers, with households accounting for around 60% of the total [1]. Besides causing considerable economic losses, food waste contributes to greenhouse gas emissions, inefficient use of natural resources, and increasing pressure on landfill capacity. In Indonesia, food waste also represents one of the largest fractions of municipal



solid waste, emphasizing the need for sustainable waste management strategies that prioritize resource recovery over conventional disposal methods [2].

At the same time, the growing demand for livestock and aquaculture production has intensified the need for sustainable protein sources. Conventional feed ingredients such as fishmeal and soybean meal remain the primary protein sources in animal diets; however, their production is associated with increasing costs, extensive land and water use, and pressure on marine resources. These challenges have encouraged the exploration of alternative feed ingredients that are nutritionally adequate while reducing environmental impacts [3].

Among the emerging approaches, insect-mediated bioconversion has received considerable attention because it simultaneously addresses two global challenges: organic waste management and sustainable protein production. Insects efficiently convert biodegradable organic materials into nutrient-rich biomass while requiring fewer natural resources than conventional livestock production. Consequently, insect farming has been recognized as a promising strategy for supporting circular economy principles and sustainable food systems [3].

Among edible insects, the yellow mealworm (*Tenebrio molitor* L.) is considered one of the most promising species due to its rapid growth, ease of mass rearing, efficient feed conversion, and favorable nutritional composition. Mealworm larvae are rich in protein, lipids, essential amino acids, vitamins, and minerals, making them suitable as alternative ingredients for livestock, poultry, and aquaculture feeds [4]. Moreover, *T. molitor* can utilize a wide range of organic substrates, enabling the conversion of biodegradable waste into valuable biomass with relatively low environmental impacts.

The nutritional quality and growth performance of *T. molitor* larvae are strongly influenced by the composition of the rearing substrate. Previous studies have demonstrated that dietary composition affects larval growth, feed conversion efficiency, nutrient deposition, and proximate composition [5]. Various agricultural by-products, cereal brans, and agro-industrial residues have been successfully used as rearing substrates, indicating that substrate selection plays an important role in determining both bioconversion efficiency and the nutritional characteristics of harvested larvae.[6]

Household food waste and seafood waste are two abundant organic residues generated from households, restaurants, and food-processing industries. Owing to their distinct nutritional characteristics, these substrates may differentially influence mealworm growth, bioconversion efficiency, nutrient composition, and frass production. However, seafood waste may also contain elevated salt concentrations and, depending on its source, may introduce undesirable contaminants such as heavy metals or pathogenic microorganisms. Therefore, feed safety should also be considered when evaluating seafood waste as a substrate for insect bioconversion. Despite the increasing number of studies on mealworm cultivation, most research has focused on artificial diets, cereal-based substrates, or agricultural by-products. Studies evaluating household food waste and seafood waste, particularly when combined as rearing substrates, remain limited. Furthermore, previous studies have generally investigated larval growth, nutritional composition, or waste reduction separately, whereas integrated evaluations that encompass bioconversion performance, larval nutritional quality, and frass characteristics remain relatively scarce [6].

In addition, although numerous studies have reported the nutritional composition of *T. molitor* larvae, relatively few have evaluated whether the resulting biomass meets established feed quality standards [8]. Comparing the nutritional composition of mealworm larvae with the Indonesian National Feed Standards (SNI) for broiler chickens, layer chickens, and fish provides a practical framework for assessing their suitability as alternative feed ingredients while identifying nutritional attributes requiring further optimization before commercial application.

Therefore, this study investigated the bioconversion of household food waste, seafood waste, and their combination using *Tenebrio molitor* larvae reared at different larval densities. The study evaluated bioconversion efficiency, larval nutritional composition through proximate analysis, and frass characteristics, followed by an assessment of the nutritional quality of the harvested larvae against the Indonesian National Feed Standards (SNI). It was hypothesized that both substrate composition and larval density would significantly influence the nutritional composition of *T. molitor* larvae, and that larvae reared on seafood waste-containing substrates would exhibit improved protein content while remaining suitable as alternative feed ingredients according to Indonesian feed standards. The findings are expected to provide scientific evidence supporting the sustainable utilization of organic waste as a valuable resource while contributing to the development of environmentally sustainable alternative feed ingredients and circular resource management.

1.2. Literature Review

1.2.1. Alternative Protein Sources and the Potential of *Tenebrio molitor*

The continuous growth of the global population, rapid urbanization, and increasing demand for livestock products have intensified the need for more sustainable protein production systems. Conventional protein sources used in animal feed, particularly fish meal and soybean meal, have long been recognized for their high nutritional quality. However, increasing production costs, fluctuating availability, competition for natural resources, and environmental impacts have raised concerns regarding their long-term sustainability. These challenges have encouraged the search for alternative protein sources that are both nutritionally valuable and environmentally sustainable [8].

Among the available alternatives, edible insects have attracted considerable attention because of their high nutritional value and relatively low environmental footprint [9]. Compared with conventional livestock production, insect farming requires less land, water, and feed while generating lower greenhouse gas emissions. In addition, several insect species can convert organic by-products and food-processing residues into valuable biomass, thereby supporting circular economy principles through nutrient recycling and waste valorization [10].

Among edible insect species, the yellow mealworm (*Tenebrio molitor* L.) is considered one of the most promising candidates for sustainable insect-based protein production because of its adaptability to various rearing substrates, ease of mass cultivation, and favorable nutritional composition [12]. Mealworm larvae are rich in crude protein, lipids, and essential amino acids, making them suitable as high-quality feed ingredients [12]. Previous studies have reported that mealworm

larvae are rich in crude protein, lipids, and essential amino acids, making them suitable as high-quality feed ingredients [12].

The promising nutritional characteristics of *T. molitor* have led to its increasing use as an alternative feed ingredient for poultry, aquaculture, and livestock production [11]. Several studies have reported that mealworm meal can partially replace fish meal or soybean meal without negatively affecting animal growth performance, feed conversion efficiency, nutrient digestibility, or overall health when included at appropriate dietary levels [11]. These findings demonstrate the potential of *T. molitor* as a sustainable protein source that could reduce dependence on conventional feed ingredients [9]. However, the nutritional quality of mealworm larvae remains highly dependent on the composition of the rearing substrate, highlighting the need to evaluate locally available organic waste streams as alternative feeding substrates.

1.2.2. Effect of Organic Waste Substrates on the Nutritional Quality of *Tenebrio molitor*

The nutritional quality of *Tenebrio molitor* larvae is closely associated with the characteristics of the organic substrates used during rearing.[13] As a detritivorous insect, mealworm larvae can utilize a wide range of organic materials, including cereal by-products, vegetable residues, fruit waste, food waste, and various agro-industrial by-products.[13] These substrates differ considerably in their protein, lipid, carbohydrate, and fiber contents, leading to differences in larval growth performance, feed conversion efficiency, and biomass composition.[14] Consequently, selecting an appropriate substrate has become a key factor in producing mealworm larvae with desirable nutritional characteristics for use in animal feed.[14]

Recent studies have shown that modifying the nutritional composition of rearing substrates can significantly alter the proximate composition of *T. molitor* larvae.[15] Larvae reared on protein-rich substrates generally contain higher crude protein levels, whereas energy-rich substrates tend to promote lipid accumulation.[13] Previous studies have demonstrated that substrate composition also influences the amino acid profile, fatty acid composition, mineral content, and moisture of mealworm larvae, indicating that adjusting substrate formulation can be an effective strategy for improving biomass quality [15] Therefore, optimizing substrate composition is considered an important approach for producing larvae with targeted nutritional profiles while maintaining satisfactory growth performance.[25]

Organic waste has emerged as a promising substrate for *T. molitor* production because it simultaneously supports insect biomass production and organic waste valorization.[16] Various organic residues, including household food waste, vegetable waste, brewer's spent grain, and former foodstuffs, have been successfully utilized as rearing substrates without adversely affecting larval performance when properly formulated.[16] The use of these organic substrates promotes resource recovery by converting low-value biodegradable materials into protein-rich insect biomass.[16] This bioconversion process also contributes to circular bioeconomy strategies by reducing the amount of organic waste disposed of in landfills while generating value-added products.[16] However, the safety and quality of organic waste substrates should also be taken into account. Specifically, seafood waste may have high salt concentrations and, depending on where it comes from, may add pollutants such pathogenic bacteria or heavy metals. Thus, to ensure the quality of insect

biomass intended for animal feed applications, appropriate substrate selection and safety evaluation are crucial.

Although numerous studies have investigated a wide range of organic substrates for mealworm production [16], relatively few have specifically evaluated seafood waste, either alone or in combination with household food waste, as rearing substrates. Furthermore, nothing is known about how different substrate combinations affect the nutritional quality of larvae in respect to national feed quality standards. Understanding these effects is important because substrate composition directly influences the nutritional value of insect biomass and ultimately determines its suitability as an alternative feed ingredient [17]. Therefore, further investigation into the use of different organic waste substrates is needed to support the development of sustainable insect-based feed ingredients with desirable nutritional characteristics.

1.2.3. Organic Waste Bioconversion Using *Tenebrio molitor*

The increasing generation of organic waste has become a major environmental challenge due to greenhouse gas emissions, inefficient resource utilization, and growing pressure on waste management systems.[18] Household food waste, agricultural residues, and agro-industrial by-products account for a large proportion of biodegradable waste generated worldwide. Conventional management practices, such as landfilling and incineration, are still widely used but often fail to recover the valuable nutrients and energy contained in these materials.[18] As a result, waste management has gradually shifted from simple disposal toward waste valorization, where organic residues are converted into value-added products through biological processes that support more sustainable resource use and environmental protection.

One promising approach to waste valorization is insect-mediated bioconversion.[18] In this process, insects utilize biodegradable organic materials as feeding substrates and convert them into protein- and lipid-rich larval biomass, nutrient-rich frass that can be utilized as an organic fertilizer, and other valuable bioproducts.[18] Compared with conventional organic waste treatment methods, insect bioconversion offers several advantages, including efficient nutrient recycling, lower greenhouse gas emissions, reduced dependence on conventional feed resources, and relatively rapid biomass production. These characteristics make insect-based bioconversion an attractive strategy for sustainable organic waste management and the development of a circular bioeconomy.[19]

The ability of *Tenebrio molitor* to utilize a wide variety of organic substrates has made this species an attractive bioconverter for organic waste streams.[19] Previous studies have shown that mealworm larvae can efficiently convert cereal by-products, vegetable residues, food waste, and other agro-industrial residues into nutritionally valuable biomass while maintaining satisfactory growth performance and feed conversion efficiency.[19] The efficiency of this process is influenced by several factors, including substrate composition, nutrient balance, carbon-to-nitrogen ratio, moisture content, temperature, relative humidity, larval density, and rearing management.[20] Therefore, careful optimization of these rearing conditions is essential to maximize waste reduction efficiency, nutrient recovery, and biomass production. Substrate safety should be taken into account

in addition to maximizing bioconversion efficiency, especially when employing seafood waste, which may contain pollutants or high salt concentrations depending on where it comes from. Both insect performance and the safety of the resulting biomass for use as animal feed may be impacted by these variables.

Beyond producing insect biomass, *T. molitor* bioconversion also generates frass, which has received increasing attention because of its potential application as an organic fertilizer [19]. Recent developments in insect biorefinery emphasize the utilization of multiple fractions of mealworm biomass, thereby improving resource efficiency and supporting circular bioeconomy strategies.[19] This integrated approach extends the role of insect farming beyond waste treatment by establishing a sustainable biorefinery platform capable of producing multiple value-added products that support circular bioeconomy strategies.[19]

Although insect-mediated bioconversion has been widely investigated using agricultural residues, cereal by-products, vegetable waste, and agro-industrial residues, relatively few studies have evaluated seafood waste, either alone or in combination with household food waste, as rearing substrates for *T. molitor*. Moreover, studies integrating bioconversion efficiency, larval nutritional composition, and compliance with national feed quality standards remain scarce. Considering the high protein and mineral contents of seafood waste, its incorporation into mixed organic substrates may alter nutrient availability during larval development, thereby influencing both waste reduction efficiency and the nutritional quality of larval biomass.[20] Therefore, evaluating the bioconversion of household and seafood waste using *T. molitor* is important for identifying substrate formulations that improve waste-reduction performance while producing nutritionally valuable biomass for sustainable animal-feed applications.

1.2.4. Nutritional Evaluation of *Tenebrio molitor* for Animal Feed According to Feed Standards

Evaluating the nutritional quality of insect-derived biomass is essential for determining its suitability as an alternative feed ingredient. Besides containing adequate levels of protein, insect biomass should also possess appropriate lipid, crude fiber, ash, and moisture contents to meet the nutritional requirements of different livestock species. Therefore, proximate analysis has become the standard method for assessing the nutritional quality of *Tenebrio molitor* larvae intended for poultry, aquaculture, and other livestock feeds. The results of proximate analysis provide a practical basis for comparing insect-derived biomass with the nutritional requirements specified for commercial animal feeds.

Previous studies have consistently reported that *T. molitor* larvae possess favorable nutritional characteristics comparable to several conventional feed ingredients. However, the proximate composition of mealworm larvae varies depending on factors such as rearing substrate, developmental stage, environmental conditions, and post-harvest processing methods. Consequently, standardized nutritional evaluation is necessary to ensure that the resulting larval biomass consistently meets the quality requirements for practical feed applications.

Rather than relying solely on proximate composition, the nutritional value of *T. molitor* larvae should also be interpreted in relation to established feed quality standards. In Indonesia, several Indonesian National Standards (SNI) specify nutritional

quality requirements for different categories of commercial animal feeds, including broiler, layer, and tilapia feeds. These standards define important nutritional parameters, such as crude protein, crude fat, crude fiber, ash, and moisture content, which serve as quality benchmarks for evaluating the suitability of feed ingredients for poultry and aquaculture production.

According to the Indonesian National Standards (SNI), broiler starter feed requires a minimum crude protein content of 20%, crude fat of at least 4%, crude fiber not exceeding 5%, ash below 9%, and moisture below 13% [21]. Layer feed requires a minimum crude protein content of 18%, crude fat of at least 3%, crude fiber below 7%, ash below 14%, and moisture below 14% [22]. For tilapia feed, the recommended nutritional composition generally ranges from 25–35% crude protein, 5–7% crude fat, crude fiber below 7%, ash below 12%, and moisture below 12%, although the exact requirements vary according to the production stage [23].

Therefore, comparing the proximate composition of *T. molitor* larvae with the Indonesian National Feed Standards provides a practical approach for evaluating their nutritional adequacy as alternative feed ingredients. Such comparisons demonstrate whether the nutritional composition of mealworm larvae meets the quality requirements for poultry and aquaculture feeds, thereby strengthening the scientific basis for their application in sustainable animal production systems. In the present study, the proximate composition of *T. molitor* larvae reared on different organic waste substrates was compared with the Indonesian National Standards for broiler, layer, and tilapia feeds to assess their potential as sustainable alternative protein sources.

Table 1. Nutritional Criteria of Indonesian Feed Standards for Broiler, Layer, and Tilapia Feeds.

Parameter	Broiler Starter Feed (SNI)	Layer Feed (SNI)	Tilapia Feed (SNI*)
Crude Protein(%)	≥20	≥18	≥25–35
Crude Fat (%)	≥4	≥3	≥5–7
Crude Fiber (%)	≤5	≤7	≤7
Ash(%)	≤9	≤14	≤12
Moisture (%)	≤13	≤14	≤12

Note: Tilapia feed values are summarized from SNI 9043-11:2024 and may vary depending on the production stage.

1.3. Research Objective

This study aimed to evaluate the effects of different organic waste substrates (household food waste, seafood waste, and their combination) and larval densities on the bioconversion performance, nutritional composition, and frass characteristics of *Tenebrio molitor* larvae. The nutritional quality of the harvested larvae was assessed through proximate composition analysis and subsequently compared with the Indonesian National Standards (SNI) for broiler, layer, and tilapia feeds to determine their suitability as alternative feed ingredients. This study also aimed to provide scientific evidence supporting the valorization of organic waste through insect-mediated bioconversion and the development of sustainable alternative protein sources for animal feed.

2. MATERIALS AND METHODS

This study was conducted under laboratory-scale conditions to evaluate the bioconversion performance, nutritional quality, and frass characteristics of *Tenebrio molitor* larvae reared on different organic waste substrates through insect-mediated bioconversion. The first factor was substrate composition, comprising household food waste (S1), a 1:1 mixture of household food waste and seafood waste (S2), and seafood waste (S3). The second factor was larval density, consisting of 0.5 larvae cm⁻² (D0.5) and 1 larvae cm⁻² (D1). Consequently, six treatment combinations with two independent biological replications (n = 2) were established, resulting in a total of twelve experimental units. The use of two biological replicates represents a limitation of the present study and should be considered when interpreting the statistical results. Future studies are recommended to include more biological replicates to improve statistical robustness.

Table 2. *Al-Ghafari*¹

Experimental Factor	Treatment	Description
Substrate Composition	S1	Household food waste
	S2	Household food waste and seafood waste
	S3	Seafood waste
Larval density	D0.5	0.5 larvae cm ⁻²
	D1	1 larvae cm ⁻²

Fresh household food waste and seafood waste were collected from local food service establishments in Surabaya, Indonesia. Before use, non-biodegradable contaminants, including plastics, paper, and other foreign materials, were manually removed. The substrates were subsequently chopped into approximately 1–2 cm pieces to facilitate larval feeding and improve substrate homogeneity. Healthy *T. molitor* larvae with relatively uniform size and developmental stage were acclimatized using wheat bran prior to the experiment and then transferred into polyethylene plastic containers measuring 21 × 14 × 15 cm. Based on the available bottom surface area (294 cm²), each container contained approximately 150 larvae for the D0.5 treatment and 300 larvae for the D1 treatment.

Throughout the 20-day experimental period, substrates were provided ad libitum to ensure that larval growth was not limited by feed availability and that the observed responses primarily reflected differences in substrate composition. Fresh substrates were replenished every four days in excess of the expected larval consumption to maintain ad libitum feeding conditions. were monitored twice daily (08:00 and 16:00) using a digital thermohygrometer throughout the 20-day experimental period to ensure suitable rearing conditions. Daily observations were conducted to record larval survival, pupation, and general rearing conditions.

At the end of the experimental period, larvae from each treatment were harvested and cleaned to remove residual substrates before proximate analysis. The nutritional composition of the larvae was determined by analyzing crude protein, crude fat, moisture, ash, and crude fiber contents using standard AOAC methods, including the Kjeldahl method for protein determination, Soxhlet extraction for crude fat, oven drying at 105°C for moisture determination, furnace incineration for ash

content, and the gravimetric method for crude fiber analysis. Carbohydrate content was calculated by difference using the following equation:

$$\text{Carbohydrate (\%)} = 100 - (\text{Crude protein} + \text{Crude fat} + \text{Moisture} + \text{Ash} + \text{Crude fiber})$$

The proximate composition obtained from each treatment was subsequently compared with the Indonesian National Standards (SNI) for broiler starter feed (SNI 8173-2:2022), layer feed (SNI 8290.5:2016), and tilapia feed (SNI 9043-11:2024) to evaluate the suitability of *T. molitor* larvae as an alternative feed ingredient. Statistical analyses were performed using Minitab Statistical Software. A two-way analysis of variance (ANOVA) was conducted to evaluate the effects of substrate composition, larval density, and their interaction on the proximate composition of *T. molitor* larvae. Prior to ANOVA, the assumptions of normality and homogeneity of variances were evaluated using the Shapiro–Wilk and Levene's tests, respectively. When significant differences were detected at $p < 0.05$, Tukey's Honestly Significant Difference (HSD) test was used to compare treatment means.

3. RESULT AND DISCUSSION

3.1. Proximate Composition of *Tenebrio molitor* Larvae

The proximate composition of *Tenebrio molitor* larvae reared on different organic waste substrates was analyzed to evaluate their nutritional quality as alternative feed ingredients. The analyzed parameters included crude protein, crude fat, moisture, ash, and carbohydrate contents. These components are widely recognized as key indicators of feed quality because they determine the nutritional value, digestibility, storage stability, and suitability of insect biomass for livestock and aquaculture feed formulations [24]. The proximate composition obtained from each treatment is presented in Table 3.

Table 3. Proximate composition (%) of *Tenebrio molitor* larvae reared on different organic waste substrates and larval densities (mean ± SD).

Treatment Code	Crude Protein (%)	Crude Fat (%)	Moisture (%)	Ash (%)	Carbohydrate (%)
S1-D0.5	29.89 ± 0.04	25.91 ± 0.03	12.25 ± 0.03	10.73 ± 0.02	21.23 ± 0.01
S1-D1	31.02 ± 0.04	26.77 ± 0.05	12.04 ± 0.11	9.61 ± 0.02	20.58 ± 0.13
S2-D0.5	31.76 ± 0.03	27.14 ± 0.02	11.68 ± 0.04	9.85 ± 0.03	19.58 ± 0.01
S2-D1	34.50 ± 0.01	24.80 ± 0.01	11.31 ± 0.02	9.67 ± 0.02	19.74 ± 0.01
S3-D0.5	36.00 ± 0.04	25.79 ± 0.06	10.10 ± 0.02	10.01 ± 0.04	18.11 ± 0.04
S3-D1	37.16 ± 0.02	24.98 ± 0.07	11.27 ± 0.03	9.60 ± 0.02	17.00 ± 0.08

As presented in Table 3, the proximate composition of *T. molitor* larvae varied among substrate treatments and larval densities. Crude protein content ranged from 29.89% in larvae reared on household food waste at a density of 0.5 larvae cm⁻² (S1-D0.5) to 37.16% in larvae reared on seafood waste at 1 larva cm⁻² (S3-D1). In contrast, carbohydrate content gradually decreased from 21.23% in S1-D0.5 to 17.00% in S3-D1. These results suggest that increasing the proportion of seafood waste in the rearing substrate was associated with higher crude protein content and lower carbohydrate content in the larvae. This finding is consistent with previous studies demonstrating that dietary composition and nutrient availability significantly influence nutrient assimilation and the proximate composition of *T. molitor* larvae [25].

Larval density also influenced the proximate composition of the larvae, although the magnitude of the response varied among substrate treatments. Across all substrate types, larvae reared at the higher density (1 larvae cm⁻²) generally exhibited slightly higher crude protein contents than those reared at the lower density (0.5 larvae cm⁻²). However, the response varied with substrate composition, suggesting a potential interaction between nutrient availability and larval density in determining nutrient deposition. Similar effects of feeding substrate and rearing conditions on the nutritional composition of *T. molitor* larvae have also been reported in previous studies [26].

Crude fat content ranged from 24.80% to 27.14%, with the highest value observed in S2-D0.5 and the lowest in S2-D1. The relatively small variation in crude fat content suggests that lipid deposition was more tightly regulated than protein accumulation under the experimental conditions, despite differences in substrate composition. Previous studies have likewise reported that changes in feeding substrates may alter lipid content, although the magnitude of variation is generally lower than that observed for crude protein [25].

Moisture content ranged from 10.10% to 12.25%, whereas ash content varied between 9.60% and 10.73%. The relatively narrow variation observed for these parameters suggests that substrate composition had a greater influence on macronutrient accumulation than on water or mineral content. Similar observations have been reported in studies evaluating different rearing substrates, where protein and lipid contents exhibited greater variability than moisture and ash contents [25].

Overall, all substrate treatments produced *T. molitor* larvae with substantial protein and lipid contents, supporting their potential as alternative feed ingredients. Larvae reared on seafood waste generally exhibited the highest crude protein concentrations, whereas the mixed substrate produced a relatively balanced protein and lipid composition. These findings agree with previous reports describing *T. molitor* as a nutritionally valuable insect whose proximate composition can be optimized through dietary manipulation [27]. The statistical significance of substrate composition and larval density on each proximate parameter is further evaluated in the following section using two-way analysis of variance (ANOVA).

3.2. Effect of Organic Waste Substrates on Larval Nutritional Composition

To evaluate the effects of substrate composition and larval density on the nutritional composition of *Tenebrio molitor* larvae, the proximate composition data were analyzed using a two-way analysis of variance (ANOVA). The analysis examined the main

effects of substrate composition and larval density, and their interaction, on the crude protein, crude fat, moisture, ash, and carbohydrate contents. The statistical results are presented in Table 4.

Table 4. Results of two-way ANOVA for the proximate composition of *Tenebrio molitor* larvae.

Parameter	Substrate	Larval density	Substrate x Density
Protein (%)	p < 0.001	p < 0.001	p < 0.001
Fat (%)	p < 0.001	p < 0.001	p < 0.001
Moisture (%)	p < 0.001	p < 0.001	p < 0.001
Ash (%)	p < 0.001	p < 0.001	p < 0.001
Carbohydrate	p < 0.001	p < 0.001	p < 0.001

The results of the two-way ANOVA demonstrated that substrate composition significantly affected all measured proximate parameters, including crude protein, crude fat, moisture, ash, and carbohydrate contents ($p < 0.001$). Larval density also significantly influenced all proximate parameters ($p < 0.001$). Furthermore, significant interactions between substrate composition and larval density were observed for all measured variables ($p < 0.001$), indicating that the effect of substrate composition on larval nutritional composition depended on larval density.

The significant effect of substrate composition indicates that differences in nutrient availability among organic waste substrates influenced the larvae's proximate composition. As shown in Table 3, larvae reared on seafood waste generally exhibited higher crude protein contents than those reared on household food waste. This trend may be attributed to the greater availability of dietary proteins and amino acid precursors in seafood waste, which can support protein synthesis during larval growth. Conversely, carbohydrate content decreased as the proportion of seafood waste increased. Because carbohydrate content was calculated by difference, increases in crude protein and other proximate fractions contributed to the lower calculated carbohydrate values observed in seafood-based treatments. Similar relationships between substrate composition and the nutritional composition of *T. molitor* larvae have been reported in previous studies.

Larval density also significantly influenced the proximate composition of the larvae. The descriptive data indicated that larvae reared at the higher density (1 larva cm⁻²) generally exhibited slightly higher crude protein contents and lower carbohydrate contents than those reared at the lower density (0.5 larva cm⁻²). However, the significant interaction detected by ANOVA indicates that the response to larval density depended on substrate composition. Higher stocking densities may alter feeding competition, nutrient availability, and feed utilization efficiency, thereby influencing nutrient assimilation and biomass composition. Similar effects of larval density on growth performance and nutrient utilization have been reported in previous studies [28]. Therefore, differences in nutrient deposition observed in the present study may also be associated with variations in feed utilization efficiency under different rearing densities.

The significant interaction between substrate composition and larval density demonstrates that the nutritional response of *T. molitor* larvae was determined by the combined influence of both experimental factors rather than by either factor independently.

This finding suggests that the same substrate may produce different nutritional outcomes depending on larval density, while the influence of larval density is likewise affected by substrate characteristics. Consequently, optimizing only one factor may not be sufficient to maximize larval nutritional quality. Instead, substrate composition and larval density should be optimized simultaneously when developing efficient mealworm-rearing systems for organic waste bioconversion.

Although seafood waste improved the crude protein content of the larvae, seafood-derived substrates may also contain elevated salt concentrations and mineral residues depending on their source. In the present study, ash content remained within a relatively narrow range across treatments, suggesting that mineral accumulation in the larvae was not substantially affected under the experimental conditions. Nevertheless, salinity, heavy metals, and microbial contaminants were not evaluated in this study. Therefore, further investigations are recommended to assess the safety aspects of seafood-waste-derived mealworm biomass before large-scale application as commercial animal feed ingredients.

Overall, the statistical analysis confirms that both substrate composition and larval density play important roles in determining the proximate composition of *T. molitor* larvae. The seafood waste treatment generally produced larvae with higher crude protein content, while the mixed substrate provided a relatively balanced nutritional composition. These findings demonstrate that optimizing both dietary composition and rearing conditions can improve the nutritional characteristics of mealworm biomass intended for sustainable livestock and aquaculture feed production. However, the statistical interpretation of the present findings should be considered in light of the limited number of biological replications ($n = 2$). Future studies employing a greater number of independent biological replicates are recommended to strengthen statistical reliability and further validate the observed treatment effects.

3.3. Comparison with Indonesian Feed Standards

The nutritional suitability of *Tenebrio molitor* larvae as an alternative feed ingredient was evaluated by comparing the proximate composition obtained in this study with the Indonesian National Standards (SNI) for broiler, layer, and tilapia feeds. Although *T. molitor* larvae are intended to be used as feed ingredients rather than complete feeds, this comparison provides a practical reference for assessing whether their nutritional composition is compatible with the nutritional requirements commonly applied in livestock and aquaculture feed formulations. The comparison is presented in Table 5 [29].

Table 5. Comparison of the proximate composition of *Tenebrio molitor* larvae with the Indonesian National Standards (SNI) for animal feeds.

Parameter	This study	Broiler SNI	Layer SNI	Tilapia SNI
Protein (%)	29.89–37.16	≥20	≥18	≥25
Fat (%)	24.80–27.14	≤7	≤7	≤10*
Moisture (%)	10.10–12.25	≤13	≤14	≤12
Ash (%)	9.60–10.73	≤9	≤14	≤15
Carbohydrate	17.00–21.23	-	-	-

As shown in Table 5, the crude protein content of *T. molitor* larvae ranged from 29.89% to 37.16%, exceeding the minimum

protein requirements specified for broiler, layer, and tilapia feeds. These results demonstrate that mealworm larvae produced from all substrate treatments provide a substantial protein source suitable for incorporation into livestock and aquaculture feed formulations. The higher protein content observed in larvae reared on seafood waste is consistent with the greater availability of dietary protein in the substrate, which may enhance protein deposition during larval growth [30].

The crude fat content ranged from 24.80% to 27.14%, exceeding the lipid levels specified for complete commercial feeds under the Indonesian National Standards. However, this does not necessarily indicate poor feed quality because whole *T. molitor* larvae naturally contain relatively high lipid concentrations. As insect biomass is generally used as a feed ingredient rather than as a complete feed, its inclusion level can be adjusted during feed formulation or the larvae can be partially defatted to achieve the desired dietary lipid content for specific livestock and aquaculture species [31].

Moisture content ranged from 10.10% to 12.25%, remaining within the maximum limits specified for broiler, layer, and tilapia feeds. Maintaining a relatively low moisture content is important because excessive moisture reduces storage stability, promotes microbial growth, and shortens feed shelf life [29]. Therefore, the moisture values obtained in this study indicate that the processed mealworm larvae possess favorable storage characteristics and are suitable for further processing as feed ingredients [31].

Ash content varied between 9.60% and 10.73%. These values complied with the requirements for layer and tilapia feeds, although some treatments slightly exceeded the maximum limit specified for broiler feeds. Ash represents the total mineral fraction of the biomass and is influenced by substrate composition and mineral accumulation during larval development [29]. Because seafood waste may contain relatively high salt and mineral contents, its use as a rearing substrate could contribute to the ash content of the harvested larvae. Although no heavy metal or salt analyses were performed in the present study, this aspect should be considered in future investigations to ensure the safety and quality of insect-derived feed ingredients produced from seafood-based substrates [30].

Unlike crude protein, crude fat, moisture, and ash, carbohydrate content is not specifically regulated in the Indonesian National Standards because carbohydrate requirements differ among animal species and are generally adjusted during feed formulation rather than through fixed quality specifications. In the present study, carbohydrate content ranged from 17.00% to 21.23%, indicating that *T. molitor* larvae also contribute an additional source of dietary energy alongside proteins and lipids [29].

Overall, the comparison with the Indonesian National Standards demonstrates that *T. molitor* larvae produced through organic waste bioconversion possess a nutritional composition suitable for use as a high-protein feed ingredient [29]. The larvae consistently exceeded the minimum protein requirements while meeting the moisture requirements specified in the standards. Although their crude fat content was higher than that of complete commercial feeds, this characteristic reflects the natural composition of whole mealworm larvae and can be accommodated through appropriate feed formulation or partial defatting [30]. These findings further support the potential of *T. molitor* larvae as a sustainable alternative protein ingredient for livestock and aquaculture feeds while simultaneously promoting

the valorization of organic waste through insect-mediated bioconversion [31].

4. CONCLUSION

This study demonstrated that both substrate composition and larval density significantly influenced the proximate composition of *Tenebrio molitor* larvae reared on organic waste substrates through insect-mediated bioconversion. Significant differences were observed in crude protein, crude fat, moisture, ash, and carbohydrate contents among the treatment combinations. Larvae reared on seafood waste generally exhibited higher crude protein contents than those reared on household food waste, with the highest protein content (37.16%) obtained from the S3-D1 treatment. Comparison with the Indonesian National Standards (SNI) indicated that the crude protein and moisture contents of the produced larvae met the nutritional requirements for broiler, layer, and tilapia feeds, supporting their suitability as a high-protein feed ingredient. Although the crude fat content exceeded the values specified for complete commercial feeds, this characteristic reflects the natural composition of whole mealworm larvae and can be managed through appropriate feed formulation or partial defatting.

Overall, the findings demonstrate that *T. molitor* larvae can efficiently convert household food waste and seafood waste into nutritionally valuable biomass while contributing to organic waste valorization and nutrient recovery. This study highlights the potential of insect-mediated bioconversion as a sustainable approach for producing alternative protein sources that support more sustainable livestock and aquaculture production systems.

However, using only two biological replicates per treatment may have limited the statistical robustness of the findings. Furthermore, only the proximate composition of the larvae was evaluated; therefore, feed-safety aspects, such as salt concentration, heavy-metal accumulation, and the microbiological quality of larvae reared on seafood-waste substrates, were not investigated. Future studies should incorporate more biological replicates, along with comprehensive safety assessments, to further validate the nutritional suitability of mealworm biomass for commercial feed applications.

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