ASEAN
Journal of Scientific and Technological Reports
Online ISSN:2773-8752

Research article

Effects of Organic Waste Substrates on Growth Performance and Nutritional Composition of Black Soldier Fly (*Hermetia illucens* L.) Larvae for Feed Production

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Citation:
Sooksoi, P.; Namee, J. Effects of organic waste substrates on growth performance and nutritional composition of black soldier fly (*Hermetia illucens* L.) larvae for feed production. *ASEAN J. Sci. Tech. Report.* **2026**, *29*(11), e263492. https://doi.org/10.55164/ajstr.v29i11.263492.

Article history:
Received: January 30, 2026
Revised: May 13, 2026
Accepted: June 20, 2026
Available online: October 10, 2026

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Abstract: Black soldier fly larvae (*Hermetia illucens* L.) have been widely studied as an alternative protein source for animal feed, and the use of organic waste substrates may enhance sustainable production of insect biomass for feed applications. This study aimed to evaluate the effects of different organic waste substrates on growth performance and nutritional composition of black soldier fly larvae. The experiment used a completely randomized design (CRD) with four treatments: commercial layer feed (control), household food waste, laying hen manure, and vegetable waste, with six replicates per treatment. Five-day-old larvae were stocked at 100 larvae per experimental unit and fed 25 g of substrate per experimental unit every two days for 28 days. The results showed that substrate type significantly affected larval growth performance and developmental traits ($p < 0.001$). Larvae reared on vegetable waste had the highest prepupal weight (2.09 g/10 larvae), pupal weight (2.29 g/10 pupae), average daily gain (0.09 g/10 larvae/day), and adult emergence, along with the lowest mortality rate (7.67%). In contrast, larvae fed laying hen manure had the highest crude protein (42.17% DM) and ash content, while those fed household food waste had the highest ether extract content (45.78% DM). These findings indicate that substrate type directly influences both growth performance and nutritional composition of larval biomass. Vegetable waste and household food waste are suitable for practical larval biomass production as alternative feed ingredients, whereas laying hen manure is more appropriate as a supplementary substrate to enhance protein and mineral contents. Therefore, substrate selection and formulation should be determined based on the intended nutritional application of larvae as animal feed ingredients.

Keywords: Black soldier fly larvae; organic waste substrates; growth performance; nutritional composition; alternative feed ingredient

1. Introduction

Currently, managing organic waste from households, fresh markets, and the agricultural sector remains a major environmental concern, as the amount of such waste continues to increase with the expansion of food production systems. If not properly managed, organic waste can cause various environmental problems, including unpleasant odors, contamination of water sources, and accumulation of greenhouse gases [1-2]. At the same time, the animal feed industry faces rising production costs because it still relies on conventional feed ingredients, which are subject to price volatility and supply

uncertainty. These circumstances have led to growing interest in reusing organic waste in recent years, particularly within the circular bioeconomy, which emphasizes converting waste into valuable resources, such as using household food waste and animal manure for animal feed production [3-4]. Black soldier fly (*Hermetia illucens* L.) is an insect widely used to bioconvert organic waste into various value-added products, particularly as a raw material for animal feed. This species grows rapidly and can accumulate substantial biomass in a short period [5]. In addition, black soldier fly larvae can utilize a wide range of organic waste substrates, including animal manure, household food waste, and plant residues [6], enabling their use as feed ingredients for various livestock species, such as poultry, pigs, and aquatic animals [7]. Owing to these characteristics, black soldier fly has attracted considerable attention as an alternative feed resource that can effectively integrate organic waste management with animal feed production [8].

However, previous studies on the use of black soldier fly larvae as animal feed ingredients have shown considerable variability in both growth performance and nutritional composition, largely depending on the type and quality of the substrates used for rearing. Different organic waste substrates vary in their chemical composition, which directly affects feeding behavior, digestion, and biomass accumulation of black soldier fly larvae. Substrates with moisture contents of 50–70% can significantly enhance larval growth and survival rates [9]. In addition, substrates with balanced protein and carbohydrate levels play an important role in increasing growth rate [10]. In contrast, substrates with excessively high fiber contents have been shown to reduce growth performance of black soldier fly larvae [11]. Moreover, substrate type also influences larval nutritional composition, as household food waste has been reported to stimulate lipid accumulation and accelerate growth rate [12]. In contrast, legume-based plant residues tend to increase protein content and essential amino acids, such as lysine, in larval biomass. Green leafy vegetable residues, on the other hand, have been associated with increased glutamic acid content in larval nutritional profiles [13]. Although several studies have reported such effects, the results remain highly variable depending on substrate type, rearing conditions, and the indicators used to evaluate growth performance and nutritional value, which limits the direct application of these findings for practical feed production.

In Thailand, commonly available organic waste substrates for rearing black soldier fly larvae include household food waste, vegetable residues from fresh markets, and animal manure from livestock farms, which are generated in large quantities year-round. Several domestic studies have reported that these waste materials can serve as alternative substrates that enhance waste degradation efficiency and promote larval growth. Household food waste has been reported to improve larval body weight and biomass accumulation [14]. Organic waste substrates have also been shown to affect larval development and biomass production [15]. Substrate type has also been reported to influence the nutrient composition of larvae and frass [16]. In addition, plant-based residues, such as spent mushroom substrates or certain vegetable wastes, have been reported to reduce the duration to the prepupal stage and increase survival rates under appropriate rearing conditions [17]. However, most studies in Thailand have focused primarily on a limited number of performance indicators, such as larval weight or growth rate. In contrast, studies that integrate multidimensional growth performance assessments with nutritional composition analysis of black soldier fly larvae reared on different organic waste substrates under systematically controlled conditions within a single experiment remain limited.

For these reasons, this study aimed to investigate the effects of different organic waste substrates on the growth performance and nutritional composition of black soldier fly larvae to provide practical information for selecting appropriate substrates for black soldier fly rearing and to support the effective utilization of organic waste in animal feed production systems.

2. Materials and Methods

2.1 Preparation of Black Soldier Fly Larvae

Black soldier fly eggs (*Hermetia illucens* L.) were kindly provided by Entonation Farm, Nanod Subdistrict, Mueang District, Phatthalung Province, Thailand. The eggs were incubated until hatching, and the resulting larvae were reared to obtain breeding stock using commercial layer feed containing 14% crude protein supplemented with soybean meal under controlled conditions of 28 ± 2 °C, 60-70% relative humidity, and a photoperiod of 12 h light:12 h dark (L:D 12:12). When the larvae reached the pupal stage, pupae were

transferred to room-temperature conditions (30-32 °C) until adult emergence. The emerged adults were then moved to a net house for mating and oviposition, and eggs were collected daily. Collected eggs were incubated at 30-32 °C and 70-80% relative humidity and reared until the larvae reached five days of age, at which point they were used for the experiment. This study was conducted under an animal use license issued in accordance with the Thai Animals for Scientific Purposes Act (No. U1-03142-2559).

2.2 Preparation of Experimental Substrates

Four types of substrates were used in this study: (1) commercial layer feed (used as the control), (2) household food waste, (3) laying hen manure, and (4) vegetable waste. Household food waste was collected from nearby households in the community surrounding the experimental site and consisted of post-consumer food residues, including cooked rice, meat scraps, and noodles. All food waste materials were thoroughly mixed to obtain a homogeneous composite sample before use as experimental substrates, and the composite sample was stored under frozen conditions throughout the experimental period. Laying hen manure was obtained from a commercial caged-layer farm. Vegetable waste used in this study consisted of discarded cabbage leaves generated from quality grading before market distribution, which represent a major and continuously available vegetable waste in the study area throughout the year. All substrates were chopped into small pieces before use. Subsequently, samples of each substrate were analyzed for chemical composition, including dry matter, crude protein, crude fiber, ether extract, ash, and nitrogen-free extract, to characterize the nutritional properties of the substrates used for rearing black soldier fly larvae, as presented in Table 1.

Table 1. Proximate composition of experimental substrates used for rearing black soldier fly larvae

Nutrient composition	Layer feed (Control, %)	Food waste (%)	Laying hen manure (%)	Vegetable waste (%)
Dry matter (DM)	86.56	31.50	30.20	7.34
Crude protein (CP)	17.54	15.40	30.80	11.54
Crude fiber (CF)	5.90	7.60	15.60	12.65
Ether extract (EE)	3.45	26.10	5.40	2.50
Ash	5.94	5.90	24.20	10.50
Nitrogen-free extract (NFE)	67.17	45.00	24.00	62.81

2.3 Experimental Design

The experiment used a completely randomized design (CRD) with four dietary treatments: commercial layer feed (control), household food waste, laying hen manure, and vegetable waste, with six replicates per treatment. Plastic containers measuring 20 × 30 × 10 cm were used as experimental units. Five-day-old black soldier fly larvae were stocked at a density of 100 larvae per container. Larvae were fed according to the assigned treatments at a rate of 25 g per container on an as-fed basis every two days, following the method described by [18]. The feeding rate was standardized on an as-fed basis to simulate practical organic waste utilization conditions. However, because the substrates differed substantially in moisture and dry matter contents, the actual dry matter intake likely varied among treatments and may have influenced larval growth performance. Estimated dry matter offered per feeding was approximately 21.64, 7.88, 7.55, and 1.84 g per container for layer feed, household food waste, laying hen manure, and vegetable waste, respectively. The experiment was conducted under controlled environmental conditions of 28 ± 2 °C, 60-70% relative humidity, and a photoperiod of 12 h light:12 h dark (L:D 12:12) for a total experimental period of 28 days from the start of the trial.

2.4 Assessment of Growth Performance and Development of Black Soldier Fly Larvae

Growth performance and developmental progression of black soldier fly larvae were monitored throughout the larval, prepupal, pupal, and adult stages as follows.

2.4.1 Measurement of Larval Body Length and Body Weight

Ten larvae were randomly sampled from each experimental unit and measured for body weight and body length at 7-day intervals from day 7 to day 28 to evaluate growth trends. Toward the end of the

experimental period, larvae within each treatment group were not always at the same developmental stage. When prepupal or pupal individuals were observed, ten individuals per replicate were randomly sampled to measure body weight and body length, and these measurements were recorded as prepupal and pupal measurements according to their respective developmental stages.

2.4.2 Growth Performance Indicators at the Prepupal Stage

Growth performance parameters at the prepupal stage included the number of larvae that reached the prepupal stage when more than 10% of the total larvae had entered this stage (NPP; individuals), the number of days required for 10% of the larvae to reach the prepupal stage (DPP; days), the body weight of larvae when 10% of the population had reached the prepupal stage (FNW; g per 10 individuals), and the average daily gain of larvae during the prepupal stage (ADG; g per 10 individuals per day).

2.4.3 Adult Emergence and Survival Assessment

When larvae and pupae from each experimental unit developed into adults, all pupae from each container were collected and incubated separately by treatment at room temperature (30-32 °C) until adult emergence. The total number of emerged adults (NTF) was recorded, and adults were classified as normal or abnormal based on the integrity of key morphological structures, including wings, legs, and body, as well as mobility. Adults with complete structures and normal movement were classified as normal adults (NNF), and the percentage of normal adults (PNF) was calculated. In addition, the percentage of developmental mortality from larval to adult stage (PDR) was calculated based on the proportion of initially stocked larvae that failed to develop into adults.

2.5 Proximate Analysis of Black Soldier Fly Larvae

Proximate analysis of black soldier fly larvae was conducted at the prepupal stage. Samples were collected from each treatment upon reaching the prepupal stage, washed with clean water, air-dried to remove surface moisture, placed in zip-lock bags, and stored at −4 °C for up to 7 days before analysis. Subsequently, 500 g of fresh larvae from each treatment were weighed and oven-dried at 60 °C for 48 h, or until constant weight was achieved. The dried samples were then ground and sieved through a 1-mm mesh screen before chemical analysis. Chemical composition analysis was performed according to the methods of the Association of Official Analytical Chemists [19], including determination of dry matter, crude protein, ether extract, crude fiber, and ash contents.

2.6 Statistical Analysis

All experimental data were subjected to analysis of variance (ANOVA) under a completely randomized design (CRD). Differences among treatment means were compared using Duncan's new multiple range test (DMRT) at a significance level of 0.05. Statistical analyses were performed using SPSS software version 29.0.1.1.

3. Results and Discussion

3.1 Growth performance and development of black soldier fly larvae

3.1.1 Effects of substrate type on larval body weight and body length

The body weight and body length of black soldier fly larvae reared on different organic waste substrates are presented in Table 2. Substrate type significantly affected larval growth performance ($p < 0.001$). Prepupal weight differed among treatments, with larvae reared on vegetable waste showing the highest mean prepupal weight (2.09 g per 10 larvae), whereas those reared on laying hen manure showed the lowest value (0.75 g per 10 larvae). Larvae reared on the layer feed (control) and household food waste treatments exhibited intermediate prepupal weights. A similar trend was observed for pupal weight. The vegetable waste treatment resulted in the highest pupal weight (2.29 g per 10 pupae), while the laying hen manure treatment produced the lowest pupal weight (1.30 g per 10 pupae) ($p < 0.001$). Significant differences were also observed in body length among treatments. Prepupal length was highest in larvae reared on vegetable waste (17.77 mm) and lowest in those reared on laying hen manure (12.86 mm) ($p < 0.001$). Likewise, pupal length followed the same

pattern, with the vegetable waste treatment yielding the greatest pupal length (21.79 mm), whereas the layer feed and laying hen manure treatments resulted in significantly shorter pupae ($p < 0.001$).

Table 2. Body weight and length of black soldier fly larvae reared on different substrates

Growth parameter	Layer feed	Food waste	Laying hen manure	Vegetable waste	P-value
Prepupal weight (g/10 larvae)	1.12 ± 0.01^{c}	1.35 ± 0.05^{b}	0.75 ± 0.05^{d}	2.09 ± 0.03^{a}	<0.001
Pupal weight (g/10 pupae)	1.56 ± 0.02^{c}	2.02 ± 0.04^{b}	1.30 ± 0.02^{d}	2.29 ± 0.06^{a}	<0.001
Prepupal length (mm)	13.05 ± 0.14^{c}	16.37 ± 0.31^{b}	12.86 ± 0.32^{d}	17.77 ± 0.92^{a}	<0.001
Pupal length (mm)	14.20 ± 0.13^{c}	18.59 ± 0.73^{b}	15.80 ± 1.02^{d}	21.79 ± 0.21^{a}	<0.001

Different superscript letters within the same row indicate significant differences at $p<0.05$ according to Duncan's new multiple range test.

The significant effects of substrate type on prepupal and pupal body weight and body length indicate that different organic waste substrates can markedly influence the growth performance of black soldier fly larvae. Larvae reared on vegetable waste exhibited the greatest body weight and length at both developmental stages, whereas those reared on laying hen manure showed the lowest values, demonstrating that substrate composition plays a critical role in larval biomass accumulation. The higher prepupal weight observed in the vegetable waste and household food waste treatments may be explained by the soft tissue structure and high moisture content of these substrates, which may enhance substrate degradation and improve nutrient availability for larval growth. These findings are consistent with previous reports indicating that substrate quality and nutrient availability strongly affect larval growth rate and developmental performance of black soldier fly larvae [10, 20]. For example, previous studies reported that larvae reared on vegetable- and fruit-based substrates exhibited greater larval weight and faster development than those reared on manure-based substrates, primarily due to higher moisture content and more readily digestible nutrients [20]. The superior growth performance observed in the vegetable waste treatment may also be associated with its substantially higher moisture content and lower dry matter concentration under the as-fed feeding system used in the present study, rather than nutrient composition alone. Previous studies have reported that substrate moisture can significantly influence larval growth and survival performance [9]. Although previous studies suggested that substrate moisture contents of approximately 50–70% are favorable for larval growth, the vegetable waste used in the present study contained substantially higher moisture content under the as-fed feeding system applied. This high moisture condition may have enhanced substrate degradation and nutrient availability, thereby contributing to improved larval growth performance. In contrast, larvae reared on laying hen manure showed the lowest body weight and body length despite the relatively high protein content of this substrate. This may be attributed to the high levels of fiber and ash in laying hen manure, which can reduce digestibility and nutrient absorption efficiency, thereby limiting biomass accumulation. Similar observations have been reported in previous studies, which found that animal manure-based substrates tend to restrict larval growth compared with softer and more easily degradable organic materials [8,16]. In addition, differences in nutrient balance among substrates, particularly the balance between protein and readily available carbohydrate sources, may also have contributed to the variation in larval growth performance observed in this study. Although laying hen manure contained relatively high crude protein levels, the substrate may have contained fewer readily available energy sources, while the elevated fiber content may have reduced nutrient utilization efficiency and biomass accumulation. In contrast, vegetable waste and household food waste may have provided more accessible carbohydrate sources and softer structural components that supported more efficient larval growth and biomass accumulation [21]. Previous studies have also suggested that microbial activity and ammonia accumulation in manure-based substrates may negatively affect larval feeding activity and growth performance [22]. High moisture conditions in vegetable waste may also support microbial degradation of the substrate and improve nutrient availability for larvae. In addition, these conditions may help maintain a more favorable microenvironment during larval development. Readily available carbohydrates from vegetable-based substrates may also provide rapid energy sources supporting larval growth and biomass accumulation. The observed consistency between prepupal and pupal weights further suggests that biomass

accumulation during the larval stage is closely associated with subsequent pupal development. This relationship supports the use of pupal weight as an indicator of larval nutrient utilization efficiency prior to metamorphosis, as reported by [14, 23]. Regarding body length, larvae reared on vegetable waste also exhibited the greatest prepupal and pupal lengths, whereas those reared on laying hen manure remained significantly shorter. These differences reflect the influence of substrate quality on morphological development, which is consistent with previous studies reporting that substrate type affects both size and body structure of black soldier fly larvae at different developmental stages [5, 12].

3.1.2 Changes in body weight and body length during the rearing period

Changes in body weight and body length of black soldier fly larvae reared on different organic waste substrates at 7, 14, 21, and 28 days of age are shown in Figure 1. Larval body weight and body length increased with age in all treatments; however, the magnitude of increase differed depending on substrate type. Larvae reared on vegetable waste consistently exhibited the highest mean body weight throughout the rearing period, with values of 0.97, 1.87, 2.09, and 2.29 g per 10 larvae at 7, 14, 21, and 28 days of age, respectively. In contrast, larvae reared on laying hen manure showed the lowest body weights at all sampling points, with corresponding values of 0.52, 0.58, 0.76, and 1.30 g per 10 larvae, respectively. A similar pattern was observed for larval body length. Larvae reared on vegetable waste showed the greatest body length at all ages, increasing from 8.62 mm at 7 days to 21.79 mm at 28 days of age. Larvae reared on household food waste exhibited intermediate growth, with body length increasing from 9.28 mm to 18.59 mm over the same period. In contrast, larvae reared on laying hen manure and commercial layer feed showed lower body lengths, although body length increased steadily with age in all treatments. Differences in larval size and morphology among treatments at the prepupal stage are illustrated in Figure 2. At 28 days of age, larvae in all treatments had developed to the pupal stage.

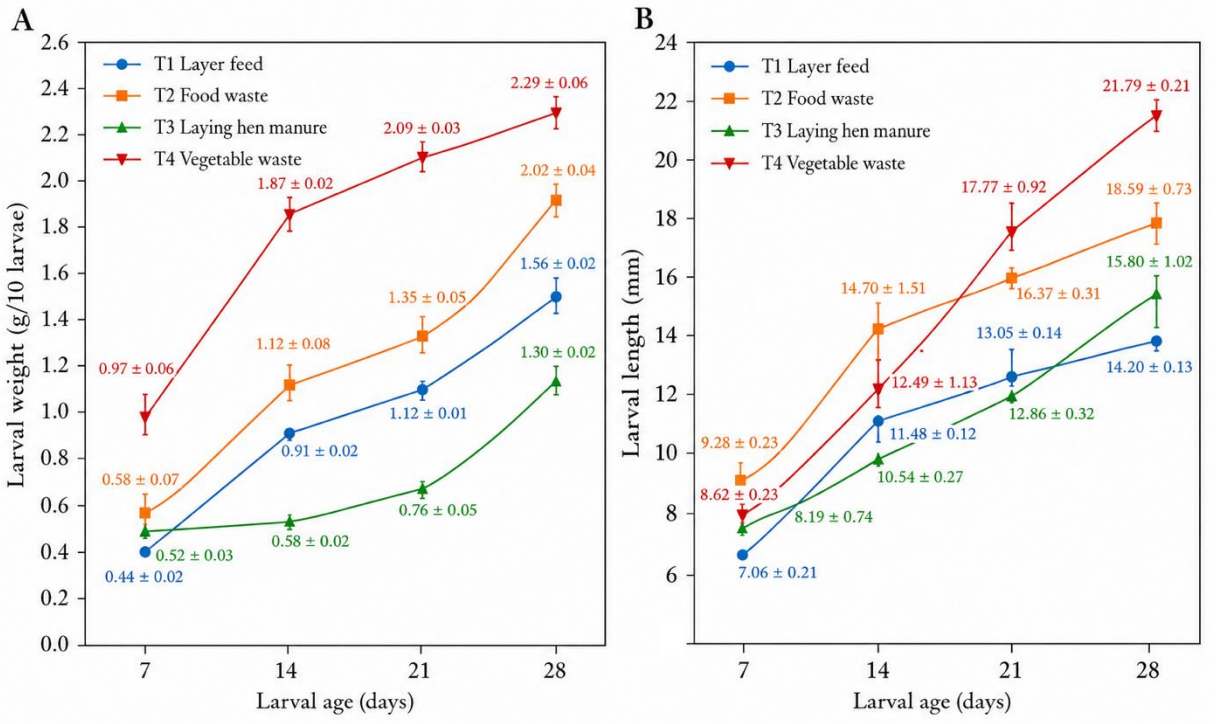


Figure 1. Mean (± SD) larval weight (A) and larval length (B) of black soldier fly larvae reared on different organic waste substrates at 7, 14, 21, and 28 days of age.

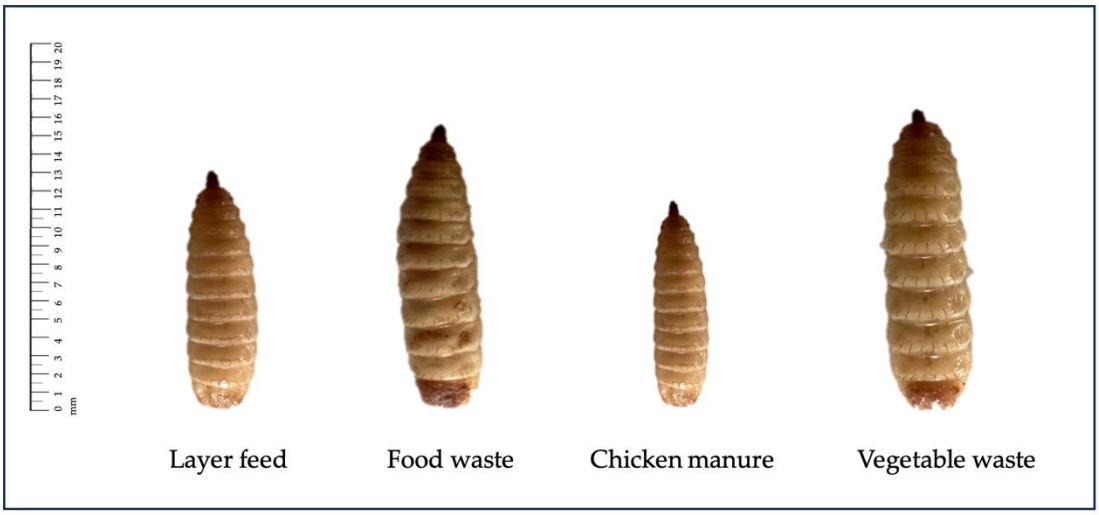


Figure 2. Representative images showing body size and morphology of black soldier fly larvae at the prepupal stage reared on different organic waste substrates. All images were captured at the same magnification. Scale (mm) is shown on the left.

The type of organic waste substrate used for rearing black soldier fly larvae significantly influenced larval growth performance, particularly in larvae reared on vegetable waste. The results of this study are comparable to those reported by [24, 25], who reared larvae on substrates composed of 50% vegetable waste, 30% apple, and 20% potato. These findings indicate that black soldier fly larvae can utilize substrates with low protein content, easily digestible carbohydrates, and high-moisture household food waste to enhance growth performance and improve the conversion of organic waste into nutrients [26]. A recent study in Thailand found that different organic waste substrates significantly affected growth performance and development in black soldier fly larvae [15]. In addition, larvae reared on laying hen manure exhibited lower body weight and smaller body size compared with those reared on commercial layer feed and vegetable waste [27], which is consistent with the findings of [28], who reported that larvae reared on fruit and vegetable waste showed better growth and faster development than those reared on poultry manure. This may be explained by the fact that, although laying hen manure may contain relatively high gross energy, it also contains ammonia produced from urea decomposition and high heat generated during microbial activity, which may be toxic to larvae at high levels and cannot be utilized by larvae [29].

3.1.3 Growth performance and developmental characteristics of black soldier fly larvae reared on different substrates

Growth performance and developmental characteristics of black soldier fly larvae reared on different organic waste substrates are presented in Table 3. Substrate type significantly affected several growth performance indicators ($p < 0.001$), except for the number of larvae entering the prepupal stage when more than 10% of the total population had reached this stage (NPP), which did not differ significantly among treatments. The number of days required for larvae to reach the prepupal stage (DPP) differed significantly among treatments, with larvae reared on vegetable waste requiring the shortest time (18.33 days), whereas those reared on laying hen manure required the longest time (23.33 days). Larvae reared on commercial layer feed and household food waste exhibited similar and intermediate DPP values. The fresh weight of larvae at the prepupal stage (FNW) differed significantly among treatments. The highest FNW was observed in the vegetable waste treatment (1.91 g per 10 larvae), followed by the layer feed and household food waste treatments, while the lowest FNW was observed in the laying hen manure treatment (0.74 g per 10 larvae). Similarly, the average daily gain during the larval–prepupal stage (ADG) was highest in larvae reared on vegetable waste (0.09 g per 10 larvae per day) and lowest in those reared on laying hen manure (0.01 g per 10 larvae per day). Regarding adult emergence, the number of normal adults (NNF) and the percentage of normal adults (PNF) differed significantly among treatments. The vegetable waste and household food waste treatments resulted in higher NNF and PNF values than the other treatments, whereas the laying hen manure

treatment produced the lowest values. In addition, the total number of emerged adults (NTF) was highest in the vegetable waste treatment (92.33 individuals) and lowest in the laying hen manure treatment (71.67 individuals). The mortality rate from the larval to adult stage (PDR) also differed significantly among treatments, with the highest mortality in the laying hen manure treatment (28.33%) and the lowest in the vegetable waste treatment (7.67%).

Table 3. Growth performance and developmental characteristics of black soldier fly larvae reared on different substrates

Growth performance	Layer feed	Food waste	Laying hen manure	Vegetable waste	P-value
NPP (larvae)	11.33 ± 0.58	13.33 ± 0.58	11.67 ± 2.08	12.00 ± 1.00	0.278
DPP (days)	20.67± 1.15^{b}	20.00 ± 1.00^{b}	23.33 ± 0.58^{a}	18.33 ± 0.58^{c}	<0.001
FNW (g/10 larvae)	1.26 ± 0.10^{b}	1.16 ± 0.22^{b}	0.74 ± 0.13^{c}	1.91 ± 0.14^{a}	<0.001
ADG (g/10 larvae /day)	0.06 ± 0.01^{b}	0.04 ± 0.02^{b}	0.01 ± 0.01^{c}	0.09 ± 0.01^{a}	<0.001
NNF (adults)	72.00 ± 1.73^{b}	85.67 ± 7.77^{a}	62.33 ± 1.53^{c}	89.33 ± 2.52^{a}	<0.001
PNF (%)	93.50 ± 0.14^{b}	96.90 ± 1.60^{a}	87.01 ± 1.62^{c}	96.77 ± 1.81^{a}	<0.001
NTF (adults)	77.00 ± 1.73^{b}	88.33 ± 6.66^{a}	71.67 ± 2.89^{c}	92.33 ± 2.89^{a}	<0.001
PDR (%)	23.00 ± 1.73^{b}	11.67 ± 6.66^{c}	28.33 ± 2.89^{a}	7.67 ± 2.89^{c}	<0.001

Different superscript letters within the same row indicate significant differences at $p<0.05$ according to Duncan's new multiple range test.

NPP = number of larvae entering the prepupal stage when more than 10% of the total population; DPP = days required for 10% of larvae to reach the prepupal stage; FNW = fresh weight of larvae at the prepupal stage (g/10 larvae); ADG = average daily gain during the larval–prepupal stage; NNF = number of normal adults; PNF = percentage of normal adults; NTF = total number of emerged adults; PDR = mortality rate from larval to adult stage.

The growth performance and developmental characteristics of black soldier fly larvae presented in Table 3 indicate that organic waste substrate type significantly influenced developmental rate (DPP), fresh weight at the prepupal stage (FNW), and average daily gain (ADG). The vegetable waste treatment produced the most favorable results for several indicators, including the shortest duration to reach the prepupal stage (DPP) and the highest FNW and ADG values, reflecting higher efficiency in converting substrates into larval biomass compared with other substrate types. In contrast, the laying hen manure treatment resulted in the longest DPP and the lowest FNW and ADG values, indicating limitations of this substrate for larval growth, despite its relatively high gross energy content [30, 31]. Although the number of larvae entering the prepupal stage when more than 10% of the population had reached this stage (NPP) did not differ significantly among treatments, this result indicates that larvae could initiate developmental progression on all substrate types. However, differences in DPP, FNW, and ADG demonstrate that substrate type plays an important role in determining growth performance and developmental characteristics. This finding is consistent with previous studies reporting that suitable substrates promote energy accumulation and physiological development in larvae before pupation [32–34], particularly with respect to developmental duration [28, 33]. The superior performance observed in the vegetable waste treatment may be explained by its high moisture content and the availability of readily digestible carbohydrates and nutrients, allowing larvae to access energy rapidly, increasing growth rate, and shortening developmental time. For adult development, the vegetable waste and household food waste treatments resulted in higher numbers and percentages of normal adults (NNF and PNF), higher total adult emergence (NTF), and lower mortality from larval to adult stage (PDR). These results indicate that substrates of appropriate quality not only enhance growth performance but also promote successful metamorphosis and adult performance. This observation is consistent with previous reports indicating that larval substrate quality influences pupal development and adult characteristics of black soldier fly [35].

3.2 Nutritional Composition of black soldier fly larvae

The proximate composition of black soldier fly larvae reared on different organic waste substrates is presented in Table 4. The results showed that substrate type significantly affected the nutritional composition

of the larvae ($p < 0.05$). Dry matter (DM) content differed significantly among treatments, with larvae reared on laying hen manure showing the highest DM value (98.63%), followed by those reared on layer feed (98.02%) and household food waste (97.41%), while larvae reared on vegetable waste had the lowest DM content (95.99%). For crude protein (CP), larvae reared on laying hen manure exhibited the highest CP content (42.17%), followed by larvae reared on layer feed and vegetable waste, which showed similar values, whereas larvae reared on household food waste had the lowest CP content (35.43%). Crude fiber (CF) content also differed significantly among treatments, with larvae reared on vegetable waste showing the highest CF content (10.46%), while larvae reared on layer feed had the lowest CF content (6.65%). Larvae reared on household food waste and laying hen manure showed intermediate CF levels. In terms of ether extract (EE), larvae reared on household food waste had the highest fat content (45.78%), followed by those reared on layer feed (41.04%), whereas larvae reared on laying hen manure and vegetable waste exhibited significantly lower EE contents. For ash content, larvae reared on laying hen manure showed the highest ash value (19.13%), followed by larvae reared on vegetable waste (13.18%), while larvae reared on household food waste had the lowest ash content (2.58%).

Table 4. Proximate composition of black soldier fly larvae reared on different substrates

Substrate	Dry matter (DM, %)	Crude protein (CP, % DM)	Crude fiber (CF, % DM)	Ether extract (EE, % DM)	Ash (% DM)
Layer feed	98.02 ± 0.17^{a}	39.85 ± 1.52^{ab}	6.65 ± 0.83^{a}	41.04 ± 1.17^{b}	7.78 ± 1.19^{c}
Food waste	97.41 ± 0.27^{b}	35.43 ± 2.31^{c}	7.39 ± 0.63^{ab}	45.78 ± 1.26^{a}	2.58 ± 0.50^{d}
Laying hen manure	98.63 ± 0.51^{a}	42.17 ± 1.70^{a}	8.30 ± 0.89^{b}	19.17 ± 0.64^{c}	19.13 ± 0.74^{a}
Vegetable waste	95.99 ± 0.38^{c}	39.11 ± 1.82^{b}	10.46 ± 0.54^{c}	23.17 ± 2.10^{c}	13.18 ± 0.65^{b}
P-value	<0.001	0.013	0.001	<0.001	<0.001

Different superscript letters within the same column indicate significant differences at $p<0.05$ according to Duncan's new multiple range test.

The present study showed that the type of organic waste substrate significantly influenced the nutritional composition of black soldier fly larvae. The larvae's nutritional profile was closely associated with the nutritional characteristics of the rearing substrates [36, 37]. Regarding dry matter (DM) content, larvae reared on laying hen manure and layer feed exhibited significantly higher DM values than those reared on vegetable waste. This may be attributed to the high moisture content and soft tissue structure of vegetable waste, which increase the proportion of water retained in larval tissues and reduce DM content. Similar findings have been reported, indicating that substrates with very high moisture content can affect both larval survival and chemical composition [38]. For crude protein (CP), larvae reared on laying hen manure showed the highest CP content, which may be associated with the high nitrogen and protein-rich compounds present in poultry manure, enabling greater protein synthesis in larvae. The crude protein values observed in the present study were within the range commonly reported for black soldier fly larvae reared on organic waste substrates in previous reviews [36, 37]. However, when considered together with growth performance results (Tables 2–3), it is evident that although the laying hen manure treatment yielded higher CP content, it resulted in lower growth performance and survival rates. This suggests that protein concentration alone is insufficient to support optimal larval development without adequate energy supply and readily digestible carbohydrates, which are essential for growth and metamorphosis [36]. In terms of ether extract (EE), larvae reared on household food waste accumulated the highest fat content, which corresponds to the typical composition of household food waste that often contains high levels of lipids and easily digestible carbohydrates. This may promote greater lipid deposition in larval tissues. Previous studies have reported that lipid content is the most variable component in black soldier fly larvae in response to changes in substrate type [36,37], which may explain why larvae reared on household food waste exhibited higher EE but lower CP than larvae reared on other substrates in this study. The large variation in ether extract content observed among substrates in the present study (19.17–45.78% DM) may have important implications for feed formulation, particularly in monogastric animals where dietary lipid levels must be carefully balanced to optimize energy utilization and maintain feed quality. Therefore, substrate selection may directly influence the suitability of black soldier fly

larvae for different animal feeding applications. Previous studies have also reported that substrate type can influence amino acid and fatty acid composition in black soldier fly larvae, which are important factors affecting their suitability as animal feed ingredients [11,33,36]. Therefore, although the present study demonstrated significant differences in proximate composition among substrates, further analysis of amino acid and fatty acid profiles would provide a more comprehensive evaluation of feed quality and nutritional value for specific livestock applications. For crude fiber (CF), larvae reared on vegetable waste showed the highest CF content. Although black soldier fly larvae are capable of degrading plant materials through enzymatic activity and associated microbial communities, substrates with high fiber content still contribute to increased structural components in larval biomass. This is consistent with recent findings indicating that plant-based substrates tend to increase structural components in larval tissues [37]. Regarding ash content, larvae reared on laying hen manure had significantly higher ash levels than those in other treatments. This result corresponds with the high ash content of the laying hen manure substrate (24.20%), leading to greater accumulation of minerals in larval tissues and consequently higher ash content in larval biomass. This finding is consistent with previous reports indicating that ash content in black soldier fly larvae is strongly associated with the mineral composition of the rearing substrate [39].

4. Conclusions

This study aimed to evaluate the effects of different organic waste substrates on the growth performance and nutritional composition of black soldier fly larvae (*Hermetia illucens* L.). The results showed that substrate type significantly affected both growth performance and the larvae's nutritional quality. Larvae reared on vegetable waste exhibited the best growth performance and adult emergence, whereas larvae fed household food waste showed the highest lipid accumulation. In contrast, larvae reared on laying hen manure had higher crude protein and ash contents but exhibited lower growth performance and survival rates than those reared on other substrates. These findings indicate that the selection of organic waste substrates for black soldier fly rearing should consider not only biomass yield and developmental performance but also the nutritional quality of larvae in relation to their intended use as animal feed ingredients. Under the conditions of this study, vegetable waste and household food waste showed high potential for practical larval biomass production, while laying hen manure is more suitable as a supplementary substrate to enhance protein and mineral contents when used in combination with other substrates. However, using manure-based substrates for insect production should also consider potential microbiological and heavy-metal safety concerns associated with animal feed applications. Therefore, practical application of these results should focus on selecting and formulating organic waste substrates according to production objectives and local substrate availability to achieve both satisfactory larval productivity and desirable nutritional composition. However, this study was conducted under controlled conditions using single substrates in each treatment, and detailed nutritional components such as amino acid and fatty acid profiles were not analyzed, which may limit the evaluation of suitability for specific animal feed formulations. In addition, the feeding rate was standardized on an as-fed basis rather than on a dry matter basis; thus, the observed differences may partly reflect moisture-driven effects associated with practical as-fed feeding conditions. Furthermore, the present study did not evaluate waste reduction efficiency or substrate conversion ratio, which may limit assessment of waste valorization efficiency among different substrates. Future studies should therefore investigate optimal substrate combinations, pre-treatment methods to improve substrate quality, waste reduction efficiency, substrate conversion ratio, and economic feasibility, including cost–benefit analysis, to support large-scale and sustainable application at community and commercial levels.

5. Acknowledgements

The authors gratefully acknowledge all individuals who provided support and cooperation during this study.

Author Contributions: Conceptualization, P.S. and J.N.; methodology, P.S. and J.N.; validation, P.S. and J.N.; formal analysis, P.S.; investigation, P.S. and J.N.; data curation, P.S. and J.N.; writing—original draft preparation, P.S.; writing—review and editing, J.N.; visualization, J.N.; supervision, J.N.; project

administration, J.N.; funding acquisition, J.N. All authors have read and agreed to the published version of the manuscript.

Funding: This project was supported by Valaya Alongkorn Rajabhat University under the Royal Patronage, Pathum Thani Province (Contract number VRU-FF68/003). The APC was funded by Valaya Alongkorn Rajabhat University under the Royal Patronage.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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