

# Influence of Growth and Protein Content of Black Soldier Fly Larvae (*Hermetia Illucens* L.) Reared with Local Fruits in Ban So Village, Mae Na Ruea, Phayao, Thailand

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This study aims to evaluate the growth rate and protein content of black soldier fly larvae reared on various diets, including pumpkin: rice bran, Namwa banana: rice bran, mango: rice bran, and a mixed fruit: rice bran formulation. Additionally, the study assessed the average light intensity, temperature, relative humidity within a greenhouse, and the moisture content of the feed. The experiment comprised four treatment groups, each containing 50 larvae, with five replicates per group. Larval growth was monitored by measuring body length and weight at 10, 17, and 24 days of age to calculate average values. Environmental parameters—light intensity, temperature, relative humidity—and feed moisture were recorded at the same intervals. Protein content in the larvae was determined using the Kjeldahl method. The results indicated an average light intensity of  $1,420.14 \pm 642.36$  lux, an average relative humidity of  $80.86 \pm 6.44\%$ , an average temperature of  $28.35 \pm 1.52^\circ\text{C}$  within the greenhouse, and a feed moisture content of  $82.24 \pm 5.25\%$ . Among the different dietary treatments, larvae fed the mixed fruit diet exhibited the greatest body length ( $18.30 \pm 3.57$  mm), while those fed the pumpkin-based diet achieved the highest body weight ( $7.72 \pm 4.32$  g). Furthermore, larvae consuming the pumpkin diet attained the highest protein content ( $40.87 \pm 0.87\%$ ). These findings suggest that pumpkin-based diets promote superior growth and protein accumulation in black soldier fly larvae compared to other dietary formulations.

**Keywords:** Black Soldier Fly Larvae, Survival Rate, Protein Content, Ban So, Phayao.

## Introduction

*Hermetia illucens* L., commonly known as the black soldier fly (BSF), belongs to the family Stratiomyidae within the order Diptera. BSF larvae have attracted global scholarly and industrial interest due to their broad applicability across agricultural, biological, food science, natural product, chemical, pharmaceutical, and environmental disciplines, including their emerging relevance in sustainable aquaculture (Purkayastha & Sarkar, 2021). This growing attention is primarily attributed to their capacity for sustainable resource generation and efficient biodegradation of organic waste, such as vegetables, fruits, and general food refuse, thus serving as a highly effective solution for managing organic matter. Several studies (Amrul et al., 2022; Mahmood et al., 2020; Salam et al., 2022) have highlighted the pivotal role BSF larvae play in producing insect-derived bioactive compounds including chitin, lipids, and proteins. These compounds offer promising alternatives for addressing global issues such as food insecurity, malnutrition, and environmental degradation associated with conventional livestock farming—particularly due to their contribution to greenhouse gas and CO<sub>2</sub> emissions (Mohan et al., 2022; Nekrasov et al., 2022). Owing to their high protein content, BSF larvae are widely applied in agricultural, biological, and pharmaceutical contexts. Traksele et al. (2021) demonstrated that these larvae also contain high-density lipoproteins, which He et al. (2024) identified as suitable alternative protein sources, such as fishmeal, frogmeal, chickenmeal, and birdmeal (Tippayadara et al., 2021).

To ensure sustainable rearing, BSF larvae should be cultivated using low-cost, environmentally sound methods that reduce greenhouse gas emissions and minimise water consumption across industries. Their by-products also contribute to soil restoration through the generation of organic fertilisers, thereby reducing dependence on chemical alternatives (Rehman et al., 2023). External environmental factors significantly influence larval development parameters such as growth rate, survival rate (%), feed intake, feed efficiency, and feed conversion ratio. For instance, optimal growth is observed at temperatures between  $26\text{--}40^\circ\text{C}$ , with peak feeding performance around  $35^\circ\text{C}$  (Singh et al., 2022). Consequently, BSF cultivation does not necessitate temperature-controlled facilities, reducing operational costs. The nutritional quality of larvae is affected by both dietary composition and environmental conditions including temperature, humidity, light, and food type. Elevated temperatures may shorten the larval life span, whereas starch and sugar diets enhance longevity, and protein-rich diets promote reproductive performance.

Mature larvae contain notable levels of protein, omega fatty acids, fibre, calcium, and lauric acid, making them ideal for inclusion in various animal feeds. During their life cycle, the protein and fat content vary: larvae (1–14 days) contain protein ranging from 38–56% and fat between 4–28%; pre-pupae contain 40.2–40.4% protein and 24.2–28.0% fat; pupae possess 43.8–46.2% protein and 7.2–8.2% fat. According to Mlambo et al. (2023), male adults contain 44.0% protein and 32.2% fat, whereas females contain 43.8% protein and 30.6% fat. Drying larvae, typically in ovens, is critical in producing high-protein animal meals, significantly reducing the reliance

on soybean feed. This processing yields a final product with over 45% protein, 35% fat, and an energy value of approximately 2,900 kcal/kg. Additionally, the processed product has antimicrobial properties, offering resistance against various pathogens including viruses, bacteria, fungi, and parasites, making it a marketable commodity valued at approximately 500 THB/kg, while dried larvae may fetch between 1,200–1,400 THB/kg (Bana et al., 2023). Currently, BSF larvae are widely acknowledged as a sustainable alternative energy source for the agriculture and food sectors. Their ability to efficiently biodegrade organic waste within roughly four weeks enables the conversion of discarded matter into nutritionally rich biomass. BSF typically contain 36–39% protein, 28–34% fat (dry matter basis), and approximately 22% calcium—higher than most other insects (Weththasinghe et al., 2021). Their high protein and glycerol content supports applications in food science and biodegradable material production, such as bio-based plastics (Nuvoli et al., 2021). In the pharmaceutical and cosmetics sectors, BSF-derived chitin—the second most abundant natural polysaccharide—has shown considerable promise in skincare and personal care products (Almeida et al., 2022; Triunfo et al., 2021), while also serving as a raw material for biodiesel production (Franco et al., 2021).

The motivation behind this research lies in the increasing global population, which has escalated food demand and underscored the urgent need for sustainable protein sources. BSF offer a viable solution, especially for the animal feed, waste management, and eco-friendly production sectors, with a considerably lower environmental impact than traditional animal protein sources. In this context, the study focused on Ban So Village in Mae Na Ruea, Phayao, Thailand, where crops such as Namwa bananas, pumpkins, and mangoes are abundantly cultivated around households and farms. Due to overproduction, surplus fruits and vegetables often become waste, creating environmental issues through decay and unpleasant odours. Prior research (Isibika et al., 2023; Mohd Rasdi et al., 2022; Ribeiro et al., 2022) supports the notion that such biodegradable waste, which is commonly discarded in local markets, provides a suitable substrate for BSF cultivation. Given the rapid growth and nutrient recycling capacity of BSF, this study sought to evaluate the comparative impact of different local food sources—including Namwa bananas and pumpkins—on larval development, nutrient content, and waste decomposition efficacy.

## Materials and Methods

### Materials

#### *Materials and Equipment used in Breeding the Black Soldier Fly Larvae*

This study utilised experimental materials and equipment to breed black soldier fly larvae, using agricultural waste fruits sourced from the market in Ban So Village, Mae Na Ruea, Phayao, Thailand. The selected fruits included Namwa bananas, pumpkins, and mangos. The equipment used in the setup comprised plastic baskets, wooden sheets, rubber bands, PVC pipes, netting, plastic tanks, plastic sheets (size 1×1, quantity: 8 sheets), needles, a digital scale, knives, glass plates, and rice bran.

#### *Equipment and Chemicals used to Test Proteins in Food and the Black Soldier Fly Larvae*

The laboratory experiment will test the protein content in Namwa bananas, pumpkins, mangos, and black soldier fly larvae using a Kjeldahl Nitrogen Analyzer. The chemicals used include sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), boric acid (H<sub>3</sub>BO<sub>3</sub>), hydrochloric acid (HCl), sodium hydroxide (NaOH), copper (II) sulfate (CuSO<sub>4</sub>), potassium sulfate (K<sub>2</sub>SO<sub>4</sub>), methyl red, bromocresol green, deionized water (DI), and dissolved oxygen (DO).

#### *Equipment used to Collect Experimental Results*

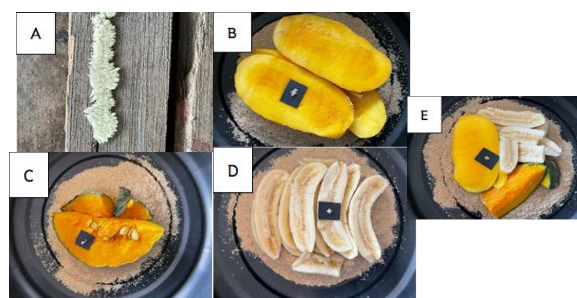
Field tools used to measure external conditions influencing the growth rate and protein content of black soldier fly larvae include a digital vernier (for growth rate), an Olympus microscope (for physiological observation), a light meter (for light intensity), a glass thermometer (for temperature), a hygrometer (for relative humidity), and a dehydrator.

#### *Population and Sample*

The experiment will divide the larvae population equally into four groups, each fed different food waste formulas: pumpkin: rice bran, Namwa banana: rice bran, mango: rice bran, and mixed fruits: rice bran, with 50 larvae per formula.

#### *Experimental Preparation*

In the first step, construct a greenhouse measuring 1.5x1.5 meters using PVC pipes, cover it with netting, and set up the environment to resemble real-life conditions. In the second step, prepare food to attract adult black soldier flies to lay eggs in a plastic tank, with a mixture of various vegetables and fruits on a plastic sheet. In the third step, set up four feeding formulas using three types of fruits: pumpkin with rice bran, Namwa banana with rice bran, mango with rice bran, and mixed fruits with rice bran. Each fruit mixture consists of 300 grams of fruit per 100 grams of rice bran in a plastic bucket. The eggs were hatched in each formula until the larvae reached 10 days of age, at which point 50 larvae from each formula were transferred. Subsequently, the larvae were fed approximately 500 grams of fruit per 100 grams of rice bran in a plastic bucket until they reached 17 and 24 days of age, when they entered the pupal stage and were suitable for protein content analysis using the Kjeldahl method. Finally, the larvae at 10, 17, and 24 days old were measured for body size and weight to calculate the average. Additionally, humidity, temperature, and light values were recorded daily, and the nutritional value was analysed (Figure 1).



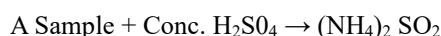
**Figure 1.** Experimental Preparation; **A:** Prepared the Black Soldier Eggs; **B:** Eggs were Reared with the Mango Formula; **C:** Eggs were Reared with the Pumpkin Formula; **D:** Eggs were Reared with the Banana Formula; **E:** Eggs were Reared with the Mixed Fruits.

## Kjeldah Method

### Digestion Test

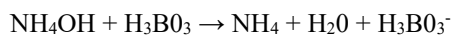
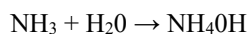
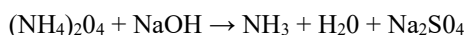
The digestion test involves the chemical analysis of samples, where each specimen is treated with concentrated H<sub>2</sub>SO<sub>4</sub> under high temperatures. Chemical catalysts such as copper(II) sulfate (CuSO<sub>4</sub>), potassium sulfate (K<sub>2</sub>SO<sub>4</sub>), sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>), and ferrous sulfate (FeSO<sub>4</sub>) are added to facilitate the conversion of nitrogen present in the sample. Upon combining the sample with these substances, ammonium sulfate ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>) is formed, as illustrated in the equation.

#### High Temperatures + Catalysts



### Distillation Test

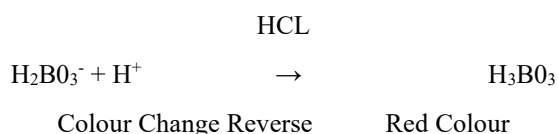
The distillation test involves the chemical elimination or modification of substances in the samples. Ammonium sulfate is eliminated using NaOH (caustic soda), an alkaline and highly corrosive base. Subsequently, the distilled ammonium reacts with H<sub>3</sub>BO<sub>3</sub>, as outlined in the equation.



Green Colour Complex

### Titration Test

H<sub>3</sub>BO<sub>3</sub>, which is bound to NH<sub>3</sub> gas, is titrated with a standard solution, such as HCl or H<sub>2</sub>SO<sub>4</sub>, to determine the total nitrogen content in the sample.



### Method of Analysis

In the initial step, approximately 1 gram of the sample (the actual weight used in the analysis) was placed onto filter paper No. 42, which was free from nitrogen. To this, 9 grams of K<sub>2</sub>SO<sub>4</sub>, 1 gram of CuSO<sub>4</sub>, and 5 glass beads were added to prevent bumping. Subsequently, concentrated H<sub>2</sub>SO<sub>4</sub> was carefully introduced, amounting to approximately 30 ml, while gently shaking the tube to ensure proper mixing in a fume hood. The samples were then placed on a digestion block at temperatures ranging from 200 to 350°C for 1 hour to ensure complete oxidation. After cooling, approximately 70 ml of deionised water (DI water) was added to the sample. The sample was then transferred to a distillation apparatus, along with a cooling bath circulator, which was activated for 45-60 minutes. Following this, a 40% NaOH solution, with a total volume of 6,000 ml, was prepared in a plastic bucket, to which reverse osmosis (RO) water was added. The NaOH solution (60 ml) was allowed to react for 5 minutes. An indicator solution was prepared by mixing 20 ml of H<sub>3</sub>BO<sub>3</sub> in a 500 ml Erlenmeyer flask, after which the

distillation test was conducted. Finally, the titration value was calculated using the standard formula, and the protein content was determined by multiplying the obtained value by the protein constant, as per the equation.

$$\text{Nitrogen (\%)} = \frac{(A - B) \times 0.014 \times N \times 100}{W}$$

A = Standard Solution used for Titration Test (ml)

B = Blank Solution (ml)

N = Concentration of Standard Solution (n)

W = Weight of the Sample (g)

(%Crude Protein) = % N × F

N = Concentration of HCL (n)

F = Factor Value (6.25)

### Statistical Data Analysis

The results of the statistical analysis were examined using SPSS (Statistical Product and Service Solutions) version 26.0. Statistical methods employed included DMRT (Duncan's Multiple Range Test) at a 95% confidence level (P<0.05) and ANOVA (Analysis of Variance).

## Results

### Environmental Factors

The environmental variables assessed included temperature, light intensity, and relative humidity, with measurements taken on seven separate occasions. These assessments were conducted at three-day intervals. Among the recorded data, the highest temperature within the breeding environment was observed during the fifth interval, reaching 30.40°C. This was followed by the third interval at 30.20°C, and the seventh at 29.00°C. In contrast, the second interval recorded the lowest temperature, measuring 26.60°C. Regarding light intensity, the maximum value was recorded in the fifth interval at 2,420 lux, with subsequent high readings of 1,980 lux during the second interval and 1,527 lux in the fourth. Conversely, the sixth interval showed the minimum light intensity of 618 lux. Relative humidity measurements indicated that the first and second intervals had the highest levels, each recording 88%. The third interval followed closely with a humidity level of 87%, while the lowest humidity was noted in the fifth interval at 74%. Furthermore, the overall averages across all measurements revealed that the mean light intensity was 1,420.14 ± 642.36 lux, the mean relative humidity stood at 80.86 ± 6.44%, and the average temperature was 28.35 ± 1.52°C. The average moisture content in the feed was found to be 82.24 ± 5.25%. These findings are summarised in Table 1.

**Table 1:** Environmental Factors in the Breeding House.

Times	The Temperature (°C)	The Light Intensity (Lux)	The Relative Humidity (%)
1	27.00	1,507	88
2	26.60	1,980	88
3	30.20	790	87
4	27.60	1,527	77
5	30.40	2,420	74
6	27.70	618	76
7	29.00	1,099	76



Regarding temperature variations across each dietary formulation, it was observed that the highest temperatures occurred during the fifth measurement for the pumpkin, Namwa banana, mango, and mixed fruit meals, registering approximately 34°C, 33°C, 30°C, and 31°C, respectively. In contrast, the lowest recorded temperatures, all measuring 26°C, were observed during the first and second intervals across all four diet types (Table 2). Additionally, the mean temperatures associated with each meal type were as follows: Namwa banana meal recorded  $29.36 \pm 3.07^\circ\text{C}$ , pumpkin meal averaged  $29.79 \pm 3.11^\circ\text{C}$ , mango meal showed  $27.07 \pm 1.68^\circ\text{C}$ , and the mixed fruit meal presented  $28.07 \pm 1.81^\circ\text{C}$ . Statistical analysis revealed a significant difference between these values ( $p < 0.05$ ) (Table 3), with temperature data further detailed in relation to the diets administered to the larvae (Table 4).

**Table 2:** The Temperature in the Formula.

Times	Pumpkin: Rice Bran	Namwa Banana: Rice Bran	Mango: Rice Bran	Mixed Fruits: Rice Bran
1	26	26	26	26
2	26	26	26	26
3	28	29	28	27
4	33	30	26	27
5	34	33	30	31
6	29	31	27	29
7	30	28	27	29

**Table 3:** The Average Temperature in the Formula.

Formula	$\bar{x} \pm \text{S.D. (Degrees Celsius)}$
Namwa Banana: Rice Bran	$29.36 \pm 3.07^*$
Pumpkin: Rice Bran	$29.79 \pm 3.11^*$
Mango: Rice Bran	$27.07 \pm 1.68^*$
Mixed Fruits: Rice Bran	$28.07 \pm 1.81$

Note: \*There is a statistically significant difference ( $p < 0.05$ ).

**Table 4:** The Temperature Statistics are in the Formula.

Formula	P. Value
Namwa Banana	Pumpkin: Rice Bran 0.654
	Mango: Rice Bran 0.020*
	Mixed Fruits: Rice Bran 0.182
Pumpkin	Namwa Banana: Rice Bran 0.654
	Mango: Rice Bran 0.006*
	Mixed Fruits: Rice Bran 0.077
Mango	Namwa Banana: Rice Bran 0.020*
	Pumpkin: Rice Bran 0.006*
	Mixed Fruits: Rice Bran 0.289
Mixed Fruits	Namwa Banana: Rice Bran 0.182
	Pumpkin: Rice Bran 0.077
	Mango: Rice Bran 0.298

Note: \*There is a statistically significant difference ( $p < 0.05$ ).

### The Growth of the Black Soldier Larvae

The weekly assessment of black soldier fly larvae growth revealed the quantity of food waste remaining after consumption of the Namwa banana, pumpkin, mango, and mixed fruit meals to be  $518.78 \pm 122.75$  grams,  $543.06 \pm 93.24$  grams,  $590.83 \pm 165.08$  grams, and  $548.45 \pm 139.83$  grams, respectively. Among these, larvae fed the mango-based diet exhibited the highest consumption rate, followed by those provided with the mixed fruit formulation. Conversely, the Namwa banana meal resulted in the lowest consumption compared to the other diets. However, statistical analysis indicated no significant difference ( $p < 0.05$ ) (Table 5). Overall, food consumption across all four diets by groups of 50 larvae ranged between

518 and 590 grams, indicating relatively similar levels of intake. The survival rate of black soldier fly larvae fed different diets indicated that the pumpkin-based meal resulted in the highest survival, with all 50 larvae surviving. This was followed by the mixed fruit diet, which yielded an average survival of  $47.17 \pm 4.40$  individuals, the mango diet at  $47.00 \pm 2.75$  individuals, and the Namwa banana diet at  $44.17 \pm 5.19$  individuals—the lowest among the four. Despite these differences, statistical analysis revealed no significant variation ( $p < 0.05$ ) (Table 6). In general, survival across all dietary groups of 50 larvae ranged between 44 and 50 individuals, reflecting relatively similar outcomes.

**Table 5:** The Average Food Intake After Eating of the Formula used to Feed the Black Soldier Larvae.

Formula	$\bar{x} \pm \text{S.D. (Grams)}$
Namwa banana: rice bran	$518.78 \pm 122.75$
pumpkin: rice bran	$543.06 \pm 93.24$
mango: rice bran	$590.83 \pm 165.08$
mixed fruits: rice bran	$548.45 \pm 139.83$

Note: \*There is a statistically significant difference ( $p < 0.05$ ).

**Table 6:** The Survival Rate.

Formula	$\bar{x} \pm \text{S.D. (Body)}$
Namwa Banana: Rice Bran	$44.17 \pm 5.19$
Pumpkin: Rice Bran	$50.00 \pm 0.00$
Mango: Rice Bran	$47.00 \pm 2.75$
Mixed Fruits: Rice Bran	$47.17 \pm 4.40$

Note: \*There is a statistically significant difference ( $p < 0.05$ ).

In addition, the assessment of the average larval body length demonstrated that larvae fed the Namwa banana diet measured  $17.13 \pm 3.17$  millimetres, those given the pumpkin meal reached  $17.56 \pm 3.05$  millimetres, the mango group attained  $18.19 \pm 3.62$  millimetres, and those consuming the mixed fruit diet achieved  $18.30 \pm 3.57$  millimetres. These differences, however, were not statistically significant ( $p < 0.05$ ) (Table 7). Regarding body weight growth, the highest value was observed in the pumpkin diet, with a mean of  $7.72 \pm 4.32$  grams, followed by the mixed fruit diet at  $7.55 \pm 4.62$  grams, and the mango diet at  $6.28 \pm 4.39$  grams. The lowest value was found in the Namwa banana diet, which had a mean of  $5.09 \pm 3.54$  grams. Statistical analysis revealed no significant differences ( $p < 0.05$ ) (Table 8).

**Table 7:** The Body Length.

Formula	$\bar{x} \pm \text{S.D. (Millimetres)}$
Namwa Banana: Rice Bran	$17.13 \pm 3.17$
Pumpkin: Rice Bran	$17.56 \pm 3.05$
Mango: Rice Bran	$18.19 \pm 3.62$
Mixed Fruits: Rice Bran	$18.30 \pm 3.57$

Note: \*There is a statistically significant difference ( $p < 0.05$ ).

**Table 8:** The Body Weight.

Formula	$\bar{x} \pm \text{S.D. (Grams)}$
Namwa Banana: Rice Bran	$5.09 \pm 3.54$
Pumpkin: Rice Bran	$7.72 \pm 4.32$
Mango: Rice Bran	$6.28 \pm 4.39$
Mixed Fruits: Rice Bran	$7.55 \pm 4.62$

Note: \*There is a statistically significant difference ( $p < 0.05$ ).

### Protein Content of the Black Soldier Larvae

The protein content in black soldier larvae reared on different fruit-based diets, including Namwa bananas, pumpkins, mangos, and mixed fruits, varied considerably.

The pumpkin diet yielded the highest protein content, with a mean of  $40.87 \pm 0.87\%$ , followed by the mixed fruit diet at  $39.84 \pm 2.16\%$ , and the Namwa banana diet at  $38.15 \pm 0.92\%$ . The mango diet exhibited the lowest protein content, at  $34.81 \pm 0.35\%$ . Statistical analysis indicated a significant difference ( $p < 0.05$ ) (Table 9), with further details provided on body weight statistics in Table 10.

**Table 9:** The Protein Content.

Formula	$\bar{x} \pm S.D. (\%)$
Namwa Banana: Rice Bran	$38.15 \pm 0.92^*$
Pumpkin: Rice Bran	$40.87 \pm 0.87^*$
Mango: Rice Bran	$34.81 \pm 0.35^*$
Mixed Fruits: Rice Bran	$39.84 \pm 2.16^*$

Note: \*There is a statistically significant difference ( $p < 0.05$ ).

**Table 10:** Statistics of the Protein Content.

Formula	P. Value
Pumpkin: Rice Bran	0.085
Namwa Banana Mango: Rice Bran	0.049*
Mixed Fruits: Rice Bran	0.230
Namwa Banana: Rice Bran	0.085
Pumpkin: Rice Bran	0.007*
Mixed Fruits: Rice Bran	60.44
Namwa Banana: Rice Bran	0.049*
Pumpkin: Rice Bran	0.007*
Mixed Fruits: Rice Bran	0.013*
Namwa Banana: Rice Bran	0.230
Pumpkin: Rice Bran	0.44
Mango: Rice Bran	0.013*

Note: \*There is a statistically significant difference ( $p < 0.05$ ).

## Discussion

### Environmental Factors

The study revealed that the relative humidity was  $80.86 \pm 6.44\%$ , the temperature was  $28.35 \pm 1.52^\circ\text{C}$ , and the humidity in food was  $82.24 \pm 5.25\%$ , consistent with the findings of Pazmiño-Palomino et al. (2022), who noted that this insect species typically inhabits tropical and subtropical regions, as well as temperate climates or hot areas. Singh et al. (2022) observed that the optimal environmental conditions for this species were a temperature of  $27^\circ\text{C}$  and relative humidity ranging from 40% to 70%. When comparing these theoretical conditions with the study results, the temperature of approximately  $28^\circ\text{C}$  and relative humidity of 80% in the present study were in line with those reported by Singh et al. (2022). Additionally, it was found that this insect requires sufficient light exposure for the proper development of its length and weight ratio, particularly during the breeding season. The larvae of the black soldier fly were reared in high-temperature conditions, which supported high survival rates. However, when temperatures exceeded  $40^\circ\text{C}$ , adult numbers decreased, and growth rates and survival ratios were negatively impacted, likely due to dehydration, high water evaporation, metabolic dysfunction, and systemic imbalances. Fazli Qomi et al. (2021) similarly found that elevated temperatures, particularly those exceeding  $40^\circ\text{C}$ , negatively affected the hatching of eggs and the transition through various life stages, including larvae, pre-pupa, pupa, and adult. They also observed that such conditions resulted in shorter lifespans, slower metabolism, and reduced growth rates.

Conversely, rearing larvae at the optimal temperature of around  $30^\circ\text{C}$  promoted faster growth, particularly in terms of body length and vital appendages, as well as improved survival and reproductive development. Thus, temperature conditions were shown to significantly influence the growth rate and reproduction of insects, with higher temperatures leading to a decline in growth rates and survival. These findings corroborate previous research, which demonstrated that environmental temperature affects various biological factors, such as survival rates, lifespan, growth rates, fertility, gender ratios, population density, and growth parameters, while also increasing the risk of disease outbreaks, including viral, bacterial, fungal, and parasitic infections (Binoy et al., 2023). Moreover, the study indicated that the light intensity within the greenhouse was  $1,420.14 \pm 642.36$  lux, and the humidity was  $80.86 \pm 6.44\%$ , conditions that were conducive to rapid egg spawning and hatching within four meals, particularly rotten fruit, after 3-4 days. The insects laid between 400-900 eggs, which aligns with the findings of Cai et al. (2022) and Kim et al. (2021), who suggested that a relative humidity range of approximately 60-70% and a light intensity of around  $135\text{--}200 \mu\text{mol}/\text{m}^2$  could promote efficient breeding during the breeding season. These insects are also resilient to extreme conditions, including high light intensity and relative humidity. Females typically lay eggs in clusters in dry areas near cracks, crevices, or decaying materials that maintain higher humidity, and the larvae hatch within approximately four days, which is the optimal time for reaching adulthood.

### The Growth of the Black Soldier Larvae

The first growth rate and survival rate across different food formulations demonstrated that the pumpkin meal significantly enhanced the survival rate, with a full  $50.00 \pm 0.00$  bodies surviving, followed by the mixed fruit meal with  $47.17 \pm 4.40$  bodies, and the mango meal with  $47.00 \pm 2.75$  bodies. In contrast, the Namwa banana meal exhibited the lowest survival rate, with only  $44.17 \pm 5.19$  bodies surviving. This is likely due to pumpkins containing higher protein content compared to other fruits, being 7.56% richer in protein than Namwa banana, mango, and mixed fruits, which may have contributed to the increased survival rate during the larvae, pre-pupal, pupal, and adult stages, achieving a 100% survival rate. This finding corresponds with the research by Ribeiro et al. (2022), who studied the effects of seven different fruit and vegetable types (wheat bran, pumpkin, apple, grape pomace, red onion, red cabbage, and spinach) at three temperature settings ( $20^\circ\text{C}$ ,  $25^\circ\text{C}$ , and  $30^\circ\text{C}$ ) and two humidity levels (natural and 70% substrate moisture). Ribeiro et al. (2022) demonstrated that pumpkins, including all parts such as peel, seeds, and pulp, contain between 1% and 2.2% protein, higher than apples and red onion. Furthermore, larvae fed with pumpkin meal exhibited good bioconversion, with feed efficiency values ranging from 14.4 to 25 and a survival rate between 81.6% and 92.4%, which was relatively high.

Regarding body length, the mixed fruit meal resulted in the longest body length at approximately  $18.30 \pm 3.57$  millimetres, followed closely by mango with an average of  $18.19 \pm 3.62$  millimetres. The pumpkin meal resulted in a

body length of  $17.56 \pm 3.05$  millimetres, while Namwa banana provided the shortest body length at  $17.13 \pm 3.17$  millimetres. The difference in body length can be attributed to the higher carbohydrate content in the mixed fruits, which included pumpkin, mango, and banana, all of which are rich in carbohydrates but lower in protein. Carbohydrates contribute to cell expansion, increasing the length of body segments and elongating the insect's limbs and abdomen during the adult phase. In terms of body weight, larvae fed on the pumpkin meal showed the highest weight at  $7.72 \pm 4.32$  grams, followed by the mixed fruit meal at  $7.55 \pm 4.62$  grams. The mango meal resulted in a weight of  $6.28 \pm 4.39$  grams, while the Namwa banana meal led to the lowest weight at  $5.09 \pm 3.54$  grams.

This research recorded that body length increased rapidly from 1.4 to 17.6 millimetres, and body weight from 44.7 to 204.6 milligrams, between Days 10 and 24, when larvae consumed the pumpkin meal. These findings align with the study by [Ribeiro et al. \(2022\)](#), who found that pumpkins contained the highest carbohydrate content (23.2%) compared to other fruits and vegetables, particularly in meals with 70% substrate moisture. Similarly, [Liu et al. \(2023\)](#) reported that body length increased from 4.0 to 16.1 millimetres, and body weight from 0.6 to 136.7 milligrams, from Day 0 to Day 11, when larvae were fed organic vegetables, leading to increased tissue development and weight gain. This study suggests that pumpkins, with their higher protein content compared to other vegetables, are more effective at promoting tissue development, while mixed fruits, despite their balanced protein content, are insufficient for optimal tissue production.

#### **Protein Content of the Black Soldier Larvae**

The analysis of different fruit-based food sources for black soldier larvae revealed varying protein content across the meals. Bananas mixed with rice bran contained approximately 3.62% protein, while pumpkins mixed with rice bran contained a significantly higher 7.56%. Mangoes mixed with rice bran offered 3.12% protein, and mixed fruits with rice bran had 5.12%. Additionally, scientific research from [Sá et al. \(2023\)](#) and [Habib et al. \(2025\)](#) reported that pumpkin seeds are rich in protein, with values ranging from 24.0% to 36.5%, averaging 33%. These findings underscore the potential of utilizing organic waste from pumpkins as a sustainable source of protein, which could help mitigate food waste in rural villages and provide a cost-effective food supplement for agricultural work, including animal feed in intensive farming environments (e.g., frog, fish, and chicken rearing). This approach could also help reduce the reliance on expensive commercial feed.

In terms of protein content, larvae consuming pumpkin meal exhibited the highest protein assimilation at  $40.87 \pm 0.87\%$ , followed by mixed fruits ( $39.84 \pm 2.16\%$ ), Namwa banana ( $38.15 \pm 0.92\%$ ), and mango meal ( $34.81 \pm 0.35\%$ ). These values are consistent with the findings of [Mlambo et al. \(2023\)](#), which reported that the crude protein levels in larvae ranged from 38% to 43% when they were fed chicken, fish, and bird meals during their 1 to 14-day life stage. Furthermore, [Hopkins et al. \(2021\)](#) demonstrated that larvae fed fish waste, particularly from *Sardinella aurita*, exhibited the highest protein content at 78.8%,

whereas those fed fruits and vegetables had a lower protein content, ranging from 12.9% to 20.1%. According to established theories, black soldier larvae fed high-protein and high-fat meals tend to exhibit higher growth rates compared to those fed lower-protein, lower-fat meals. Additionally, moderate or large amounts of nutrient-rich food lead to the production of larger pupae compared to those reared on low-nutrient diets. Therefore, the protein content of the diet plays a crucial role in the synthesis of proteins, which are essential nutrients for future consumers, such as animals and humans. This research suggests that further studies should explore the potential for enhancing protein production in black soldier larvae through the analysis of food waste and its impact on farm animal growth in agriculture. This would open up opportunities for sustainable, low-cost protein sources for animal feed and other agricultural applications.

## **Conclusion**

The study identified key environmental factors within the greenhouse that influenced the growth and development of black soldier fly larvae. The average light intensity was recorded at  $1420.14 \pm 642.36$  lux, with a relative humidity of  $80.86 \pm 6.44\%$  and an average temperature of  $28.35 \pm 1.52^\circ\text{C}$ . Additionally, the moisture content in the food provided to the larvae was  $82.24 \pm 5.25\%$ . These environmental conditions supported optimal larval development, as reflected in the performance metrics across different food formulas. The highest survival rate was observed in larvae fed the pumpkin meal, with  $50.00 \pm 0.00$  individuals surviving. The maximum average body length ( $18.30 \pm 3.57$  mm) was recorded in the group fed mixed fruits, while the highest body weight ( $7.72 \pm 4.32$  g) and protein content ( $40.87 \pm 0.87\%$ ) were achieved with the pumpkin meal. These findings suggest that the pumpkin-based diet not only enhances protein synthesis but also supports better larval growth compared to other food types. Therefore, pumpkin meals are particularly effective in promoting higher growth rates and increased protein content in black soldier fly larvae, making them a valuable dietary component for sustainable insect farming.

## **Data Availability**

The data that support the findings of this study are available on request from the corresponding author.

## **Conflicts of Interest**

The authors declare that they have no conflicts of interest.

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## **References**

- Almeida, C., Murta, D., Nunes, R., Baby, A. R., Fernandes, Â., Barros, L., Rijo, P., & Rosado, C. (2022). Characterization of lipid extracts from the *Hermetia illucens* larvae and their bioactivities



- for potential use as pharmaceutical and cosmetic ingredients. *Heliyon*, 8(5), e09455-e09455. <https://doi.org/10.1016/j.heliyon.2022.e09455>
- Amrul, N. F., Kabir Ahmad, I., Ahmad Basri, N. E., Suja, F., Abdul Jalil, N. A., & Azman, N. A. (2022). A Review of Organic Waste Treatment Using Black Soldier Fly (*Hermetia illucens*). *Sustainability*, 14(8), 4565. <https://doi.org/10.3390/su14084565>
- Bana, J. J., Barlian, A., & Ridwan, A. (2023). Production performance of kampung hens fed rations containing black soldier fly larvae powder. *Iranian Journal of Veterinary Science and Technology*, 15(3), 48-56. <https://doi.org/10.22067/ijvst.2023.79438.1204>
- Binoy, C., Delvare, G., Colombo, W. D., Surya, K. S., & Sureshan, P. M. (2023). Hymenopteran parasitoids of black soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae) in chicken farms with two new species from India. *Journal of Asia-Pacific Entomology*, 26(4), 102140. <https://doi.org/10.1016/j.aspen.2023.102140>
- Cai, M., Li, L., Zhao, Z., Zhang, K., Li, F., Yu, C., Yuan, R., Zhou, B., Ren, Z., Yu, Z., & Zhang, J. (2022). Morphometric Characteristic of Black Soldier Fly (*Hermetia illucens*) · Wuhan Strain and Its Egg Production Improved by Selectively Inbreeding. *Life (Basel, Switzerland)*, 12(6), 873. <https://doi.org/10.3390/life12060873>
- Fazli Qomi, S. m., Danaeefard, M. r., Farhang, A. b., Hosseini, S. P., & Arast, Y. (2021). Effect of Temperature on the Breeding Black Soldier Fly Larvae in Vitro for Basic Health-oriented Research. *Archives of Hygiene Sciences*, 10(1), 67-74. <https://doi.org/10.52547/archhygsci.10.1.67>
- Franco, A., Scieuzo, C., Salvia, R., Petrone, A. M., Tafi, E., Moretta, A., Schmitt, E., & Falabella, P. (2021). Lipids from *Hermetia illucens*, an Innovative and Sustainable Source. *Sustainability*, 13(18), 10198. <https://doi.org/10.3390/su131810198>
- Habib, M., Singh, S., Ahmad, S., Jan, S., Gupta, A., Jan, K., & Bashir, K. (2025). Ultrasonication modifies the structural, thermal and functional properties of pumpkin seed protein isolate (PSPI). *Ultrasonics sonochemistry*, 112, 107172-107172. <https://doi.org/10.1016/j.ultsonch.2024.107172>
- He, Y., Peng, H., Jin, M., Wang, J., Li, S., Li, M., Zhu, T., Zhang, L., Chen, X., & Zhou, Q. (2024). Application evaluation of black soldier fly (*Hermetia illucens*) larvae oil in shrimp feed: Effects on growth performance, antioxidant capacity and lipid metabolism. *Aquaculture Reports*, 36, 102174. <https://doi.org/10.1016/j.aqr.2024.102174>
- Hopkins, I., Newman, L. P., Gill, H., & Danaher, J. (2021). The Influence of Food Waste Rearing Substrates on Black Soldier Fly Larvae Protein Composition: A Systematic Review. *Insects*, 12(7), 608. <https://doi.org/10.3390/insects12070608>
- Isibika, A., Simha, P., Vinnerås, B., Zurbrugg, C., Kibazohi, O., & Lalander, C. (2023). Food industry waste - An opportunity for black soldier fly larvae protein production in Tanzania. *Science of The Total Environment*, 858, 159985. <https://doi.org/10.1016/j.scitotenv.2022.159985>
- Kim, C.-H., Ryu, J., Lee, J., Ko, K., Lee, J.-y., Park, K. Y., & Chung, H. (2021). Use of Black Soldier Fly Larvae for Food Waste Treatment and Energy Production in Asian Countries: A Review. *Processes*, 9(1), 161. <https://doi.org/10.3390/pr9010161>
- Liu, Y., Liu, J., He, J., Lu, H., Sun, S., Ji, F., Dong, X., Bao, Y., Xu, J., He, G., & Xu, W. (2023). Chronological and Carbohydrate-Dependent Transformation of Fatty Acids in the Larvae of Black Soldier Fly Following Food Waste Treatment. *Molecules (Basel, Switzerland)*, 28(4), 1903. <https://doi.org/10.3390/molecules28041903>
- Mahmood, S., Bari Tabinda, A., Ali, A., & Zurbrugg, C. (2020). Reducing the Space Footprint of Black Soldier Fly Larvae Waste Treatment by Increasing Waste Feeding Layer Thickness. *Polish Journal of Environmental Studies*, 30(1), 771-779. <https://doi.org/10.15244/pjoes/122618>
- Mlambo, V., Dibakoane, S. R., Mashiloane, T., Mukwevho, L., Wokadala, O. C., & Mnisi, C. M. (2023). Rethinking food waste: Exploring a black soldier fly larvae-based upcycling strategy for sustainable poultry production. *Resources, Conservation and Recycling*, 199, 107284. <https://doi.org/10.1016/j.resconrec.2023.107284>
- Mohan, K., Rajan, D. K., Muralisankar, T., Ganesan, A. R., Sathishkumar, P., & Revathi, N. (2022). Use of black soldier fly (*Hermetia illucens* L.) larvae meal in aquafeeds for a sustainable aquaculture industry: A review of past and future needs. *Aquaculture*, 553, 738095. <https://doi.org/10.1016/j.aquaculture.2022.738095>
- Mohd Rasdi, F. L., Ishak, A. R., Hua, P. W., Mohd Shaifuddin, S. N., Che Dom, N., Shafie, F. A., Abdullah, A. M., Abdul Kari, Z., & Atan, E. H. (2022). Growth and Development of Black Soldier Fly (*Hermetia illucens* (L.)), Diptera: Stratiomyidae) Larvae Grown on Carbohydrate, Protein, and Fruit-Based Waste Substrates. *Malaysian Applied Biology*, 51(6), 57-64. <https://doi.org/10.55230/mabjournal.v51i6.2386>
- Nekrasov, R. V., Ivanov, G. A., Chabaev, M. G., Zelenchenkova, A. A., Bogolyubova, N. V., Nikanova, D. A., Sermyagin, A. A., Bibikov, S. O., & Shapovalov, S. O. (2022). Effect of Black Soldier Fly (*Hermetia illucens* L.) Fat on Health and Productivity Performance of Dairy Cows. *Animals : an open access journal from MDPI*, 12(16), 2118. <https://doi.org/10.3390/ani12162118>
- Nuvoli, D., Montevicchi, G., Lovato, F., Masino, F., Van Der Borght, M., Messori, M., & Antonelli, A. (2021). Protein films from black soldier fly (*Hermetia illucens*, Diptera: Stratiomyidae) prepupae: effect of protein solubility and mild crosslinking. *Journal of the Science of Food and Agriculture*, 101(11), 4506-4513. <https://doi.org/10.1002/jsfa.11091>

- Pazmiño-Palomino, A., Reyes-Puig, C., & Del Hierro, A. G. (2022). How could climate change influence the distribution of the black soldier fly, *Hermetia illucens* (Linnaeus)(Diptera, Stratiomyidae)? *Biodiversity Data Journal*, 10, e90146. <https://doi.org/10.3897/BDJ.10.e90146>
- Purkayastha, D., & Sarkar, S. (2021). Sustainable waste management using black soldier fly larva: a review. *International Journal of Environmental Science and Technology*, 19(12), 12701-12726. <https://doi.org/10.1007/s13762-021-03524-7>
- Rehman, K. U., Hollah, C., Wiesotzki, K., Rehman, R. U., Rehman, A. U., Zhang, J., Zheng, L., Nienaber, T., Heinz, V., & Aganovic, K. (2023). Black soldier fly, *Hermetia illucens* as a potential innovative and environmentally friendly tool for organic waste management: A mini-review. *Waste management & research : the journal of the International Solid Wastes and Public Cleansing Association, ISWA*, 41(1), 81-97. <https://doi.org/10.1177/0734242X221105441>
- Ribeiro, N., Costa, R., & Ameixa, O. M. C. C. (2022). The Influence of Non-Optimal Rearing Conditions and Substrates on the Performance of the Black Soldier Fly (*Hermetia illucens*). *Insects*, 13(7), 639. <https://doi.org/10.3390/insects13070639>
- Sá, A. G. A., Pacheco, M. T. B., Moreno, Y. M. F., & Carciofi, B. A. M. (2023). Processing effects on the protein quality and functional properties of cold-pressed pumpkin seed meal. *Food Research International*, 169, 112876. <https://doi.org/10.1016/j.foodres.2023.112876>
- Salam, M., Shahzadi, A., Zheng, H., Alam, F., Nabi, G., Dezh, S., Ullah, W., Ammara, S., Ali, N., & Bilal, M. (2022). Effect of different environmental conditions on the growth and development of Black Soldier Fly Larvae and its utilization in solid waste management and pollution mitigation. *Environmental Technology & Innovation*, 28, 102649. <https://doi.org/10.1016/j.eti.2022.102649>
- Singh, A., Marathe, D., & Kumari, K. (2022). Black Soldier Fly *Hermetia illucens* (L.): Ideal Environmental Conditions and Rearing Strategies. *Indian Journal of Entomology*, 1-11. <https://doi.org/10.55446/ije.2022.166>
- Tippayadara, N., Dawood, M. A. O., Krutmuang, P., Hoseinifar, S. H., Doan, H. V., & Paolucci, M. (2021). Replacement of Fish Meal by Black Soldier Fly (*Hermetia illucens*) Larvae Meal: Effects on Growth, Haematology, and Skin Mucus Immunity of Nile Tilapia, *Oreochromis niloticus*. *Animals : an open access journal from MDPI*, 11(1), 193. <https://doi.org/10.3390/ani11010193>
- Traksele, L., Speiciene, V., Smicius, R., Alencikiene, G., Salaseviciene, A., Garmiene, G., Zigmantaite, V., Grigaleviciute, R., & Kucinskas, A. (2021). Investigation of in vitro and in vivo digestibility of black soldier fly (*Hermetia illucens* L.) larvae protein. *Journal of Functional Foods*, 79, 104402. <https://doi.org/10.1016/j.jff.2021.104402>
- Triunfo, M., Tafi, E., Guarnieri, A., Scieuzo, C., Hahn, T., Zibek, S., Salvia, R., & Falabella, P. (2021). Insect Chitin-Based Nanomaterials for Innovative Cosmetics and Cosmeceuticals. *Cosmetics*, 8(2), 40. <https://doi.org/10.3390/cosmetics8020040>
- Weththasinghe, P., Hansen, J. Ø., Mydland, L. T., & Øverland, M. (2021). A systematic meta-analysis based review on black soldier fly (*Hermetia illucens*) as a novel protein source for salmonids. *Reviews in Aquaculture*, 14(2), 938-956. <https://doi.org/10.1111/raq.12635>