ESTABLISHING AN INTENSIVE BLACK SOLDIER FLY (*HERMETIA ILLUCENS*) FARMING SYSTEM AS A PROTEIN SUPPLEMENTATION FOR SMALLSCALE POULTRY PRODUCTION

MUHAMMAD KASYFI BIN MOHD YAZID

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CERTIFICATION

This is to certify that we have read this research paper entitled **'Establishing An Intensive Black Soldier Fly (*Hermetia Illucens*) Farming System As A Protein Supplementation For Smallscale Poultry Production'** by Muhammad Kasyfi Bin Mohd Yazid, and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course DVT 55204 – Research Project.

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Dr Mohd Farhan Hanif Bin Reduan

DVM (UMK), PhD (UPM)

Senior Lecturer,

Faculty of Veterinary Medicine

Universiti Malaysia Kelantan

(Supervisor)

Dr Abd Rahman Bin Aziz

DVM (UAF), MSc (University of Melbourne, Australia),

Senior Fellow,

Faculty of Veterinary Medicine

Universiti Malaysia Kelantan

(Co-supervisor)

Mr Amirul Faiz Bin Mohd Azmi

Master in Animal Nutrition (UPM)

Lecturer,

Faculty of Veterinary Medicine

Universiti Malaysia Kelantan

(Co-supervisor)

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Thank You

DEDICATIONS

I write my dissertation as a tribute to my numerous friends and family. My loving parents, Anisa Binti Ali and Mohd Yazid Bin Awalludin, whose words of support and encouragement to keep going keep ringing in my ears, deserve to be thanked.

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ABBREVIATION

BSFL	-	Black Soldier Fly Larvae
BSF	-	Black Soldier Fly
CP	-	Crude protein
DOL	-	Day of Larvae
kg	-	Kilogram
g	-	Gram
L	-	Litre
cm	-	Centimetre
g	-	Gram
df	-	Degree of Freedom
Sig.	-	*p*-value

ABSTRACT

An abstract of the research paper presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement on the course DVT 55204 – Research Project.

Black soldier fly larvae (BSFL) has been proposed as alternate protein sources to maise and soybean meals and chicken feed additives. An intensive Black Soldier Fly (BSF) intensive farming method was studied to determine its suitability for smallscale poultry farming in Malaysia. During the production of BSFL, the growth performance and nutrient composition of BSFL were assessed. The treatment groups for the production of BSFL were divided into three different feed substrate groups: (A) chicken manure with fruit wastes served as the control group, (B) Bokashi protein meal with fruit wastes and (C) soybean meal with fruit waste served as the experimental groups. One gram of 5-day-old BSF eggs was placed in each feeding substrate group, and growth performance was monitored for 20 days. Several parameters were measured, including the weight, length, height, and width of the BSFL. The data were recorded every three days until the larvae reached two and a half weeks. The sand roasting method was implemented to dry the BSF larvae, and nutritional analysis was carried out using standard proximate analysis. Results showed BSF larvae supplemented with Bokashi protein had the highest growth performance compared to other groups. The nutritional findings of BSF larvae that fed with Bokashi meal showed 21.0 g/100g of crude protein, 25.5 g/100g of total fat and 10.6 g/100g of carbohydrate. Meanwhile, the moisture content at 34.1 g/100g, 10.2 g/100g of crude fiber, and 8.8 g/100g of total ash. The nutritional analysis revealed the crude protein and crude fat were regarded as low due to improper drying method. In conclusion, this study demonstrated that the larvae's growth performance were unaffected ($p > 0.05$) by the various feeding

substrates provided. A proper drying method could obtain a high yield of dry matters of BSFL.

***Keywords**: Black Soldier Fly, Black Soldier Fly larvae, intensive farming, growth performance, nutritional analysis*

ABSTRAK

Abstrak kertas penyelidikan ini dibentangkan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan, sebagai keperluan sebahagian daripada kursus DVT 55204 – Projek Penyelidikan.

Larva lalat askar hitam (BSFL) telah dicadangkan sebagai sumber protein alternatif kepada makanan jagung dan kacang soya serta bahan tambahan makanan ayam. Kaedah penternakan intensif Black Soldier Fly (BSF) telah dikaji untuk menentukan kesesuaiannya untuk penternakan ayam kecil di Malaysia. Semasa pengeluaran BSFL, prestasi pertumbuhan dan komposisi nutrien BSFL telah dinilai. Kumpulan rawatan untuk pengeluaran BSFL dibahagikan kepada tiga kumpulan substrat makanan yang berbeza: (A) baja ayam dengan sisa buah dijadikan sebagai kumpulan kawalan, (B) Makanan protein Bokashi dengan sisa buah dan (C) makanan kacang soya dengan sisa buah yang dihidangkan. sebagai kumpulan eksperimen. Satu gram telur BSF berusia 5 hari diletakkan dalam setiap kumpulan substrat pemakanan, dan prestasi pertumbuhan dipantau selama 20 hari. Beberapa parameter telah diukur, termasuk berat, panjang, tinggi, dan lebar BSFL. Data direkodkan setiap tiga hari sehingga larva mencapai dua setengah minggu. Kaedah pemanggangan pasir dilaksanakan untuk mengeringkan larva BSF, dan analisis pemakanan dijalankan menggunakan analisis proksimat standard. Keputusan menunjukkan larva BSF yang ditambah dengan protein Bokashi mempunyai prestasi pertumbuhan tertinggi berbanding kumpulan lain. Penemuan khasiat larva BSF yang diberikan makanan protein Bokashi menunjukkan 21.0 g/100g protein kasar, 25.5 g/100g jumlah lemak dan 10.6 g/100g karbohidrat. Manakala, kandungan lembapan pada 34.1 g/100g, 10.2 g/100g gentian kasar, dan 8.8 g/100g jumlah abu. Analisis pemakanan mendedahkan protein mentah dan lemak mentah dianggap rendah kerana kaedah pengeringan yang tidak betul. Kesimpulannya, kajian ini menunjukkan bahawa prestasi pertumbuhan larva tidak terjejas ($p > 0.05$) oleh pelbagai substrat pemakanan yang disediakan. Kaedah pengeringan yang betul boleh memperoleh hasil yang tinggi bagi bahan kering BSFL.

***Kata kunci**: Lalat Askar Hitam, larva, penternakan intensif, prestasi pembesaran, analisis nutrien*

1.0 INTRODUCTION

Poultry is by far the most common type of livestock worldwide. However, with the increased human population, which is anticipated to reach 9 billion by 2050, animal protein consumption is expected to rise shortly. Furthermore, global warming and climate change may affect the quality and quantity of animal feedstuffs such as maise and soybean meal (Abd El-Hack *et al*., 2020). Soybean meal, for example, is a common protein source in chicken feed compositions. Nevertheless, in recent years, the high cost of this part has become a significant concern for the long-term economic viability of chicken production, especially in developing countries. The increasing loss of arable land poses a global problem. These challenges urge innovative solutions to discover the most sustainable path feasible for global food production. Alternative feed supplies must be investigated in these situations (Abd El-Hack *et al*., 2020).

Black soldier fly larvae (BSFL) have been proposed as alternative protein sources to soybean and maise meals and chicken feed additives. The larvae are commercially farmed on cereal by-products because they do not absorb pesticides or mycotoxins (Kawasaki *et al*., 2019). Black soldier flies have also not been reported as a pest or vector of disease-causing pathogens (Tomberlin *et al.*, 2009; Diener *et al*., 2011). Surprisingly, the larval stage has been shown to reduce the amount of organic waste on organic compost and the number of pathogens like *E. coli* and *Salmonella enteric* serotype *enteritidis* (Erickson *et al*., 2004; Liu *et al*., 2008; Bullock *et al*., 2013). The insect larvae help diminish the population of harmful insects, pests or carriers of common disease-causing diseases, such as the common housefly (*Musca domestica*), in human and domesticated animal habitats (Bradley & Sheppard, 1984). Also, BSF larvae fed animal manure and organic waste as animal feed could be a long-term way to recycle waste resources that have yet to be used. Besides, chitin, an indigestible compound, has been discovered in the BSF larvae diet. When something that cannot be digested gets into the cecum of a laying hen, it changes the gut bacteria and stops short-chain fatty acids from being made. The hens' cecal microbiome changed when BSF larvae feed was given to laying hens instead of soybean meal (Kawasaki *et al.*, 2019).

The Black Soldier Fly (*Hermetia illucens*) belongs to the Stratiomyidae family of true flies (Diptera). Though it originated in the Americas, it today thrives in tropical and temperate climates worldwide (Čičková *et al*., 2015). The adults BSFL only drink water, do not approach humans, do not bite or sting, and do not transmit or spread diseases (Čičková *et al*., 2015). Black soldier fly larvae (BSFL) have been employed in smallscale waste management applications utilising substrates such as manure, rice straw, food waste, grains, faecal sludge, animal offal, kitchen debris, and other organic materials (Wang & Shelomi, 2017). They may be the most diverse substrates they can digest (Kim *et al.*, 2011). Their larval growth duration is more than three weeks, compared to five days for flies like house and carrion flies, implying that a single larva will eat more substrate and generate larger pupae (Čičková *et al*., 2015). Furthermore, when BSFLs are at the pre-pupa stage, they will intuitively leave the substrate and travel to a high, clean location, a behaviour known as "self-harvesting," which eliminates a labour-intensive phase of their farming process (Craig Sheppard *et al*., 1994). These advantages make BSFL a viable livestock feed (Wang & Shelomi, 2017).

2.0 PROBLEM STATEMENT

i. There is limited information on the Black Soldier Fly (BSF) intensive farming system in Malaysia.

ii. There is inadequate information on the effect of feeding different substrates on the growth performance and nutritional values of BSF larvae.

3.0 RESEARCH HYPOTHESIS

i. The appropriate farming method of Black Soldier Fly for utilization in small-scale poultry production is intensive farming.

ii. Different feeding substrate affect the growth performance and nutritional values of Black Soldier Fly larvae.

4.0 RESEARCH QUESTIONS

i. What is the appropriate farming method of Black Soldier Fly for utilisation in small-scale poultry production?

ii. Do different feeding substrates affect the growth performance and nutritional values of Black Soldier Fly Larvae?

5.0 RESEARCH OBJECTIVE

i. To establish a farming method of BSF for utilisation in small-scale poultry production.

ii. To evaluate the growth performance and nutritional value of BSF larvae with different feeding substrates.

6.0 LITERATURE REVIEW

6.1 Black soldier fly life cycle

Hermetia illucens, the Black Soldier Fly, belongs to the dipteran family Stratiomyidae. It may be found in tropical and sub-tropical places all over the world. The egg begins the BSF life cycle simultaneously, signalling the end of the previous life stage: a clutch of eggs laid by a fly (also called ovipositing). In tiny, dry, protected crevices, the female fly lays a bundle of 400 to 800 eggs among decaying organic waste (Dortmans *et al.*, 2021). The female dies shortly after laying the eggs. Since the eggs are close to the decaying organic matter, the larvae will have food close by when they hatch. The safe chambers keep predators from getting to the eggs and keep the sun from drying out the egg packets. The eggs hatch in four days on average, and the larvae emerge after that. The larvae are cream-coloured and develop from a few millimetres to roughly 2.5 cm in length and 0.5 cm in breadth, feeding voraciously on decaying organic waste (Dortmans *et al.*, 2021).

The larvae will require 14-16 days to grow under optimum conditions with excellent diet quality and quantity. On the other hand, the BSF larva is a hardy creature that can continue its life cycle even in adverse conditions. Because the BSF primarily consumes during the larval stage, adequate fat stores and protein are saved throughout this period of larval development to allow the larvae to pupate, emerge as flies, locate partners, copulate, and (as a female) produce eggs before dying (Dortmans *et al.*, 2021). After passing through five larval stages, the larvae reach the last larval stage, the prepupa. The larva replaces its mouthpart with a hook-shaped structure and changes colour from dark brown to charcoal grey as it transforms into a prepupa. It utilises this hook to migrate out and away from the food source to a nearby dry, humus-like, shady, and sheltered area that it considers secure from predators, and where the imago emerges from the pupa and flies away with no difficulty (Dortmans *et al.,* 2021).

The transformation of a pupa into a fly is known as pupation. The pupation stage begins when the prepupa finds an appropriate position and becomes inflexible and rigid. It is optimal for successful pupation if the ambient circumstances do not vary too much or if they stay warm, dry, and shaded. The pupation period lasts around two to three weeks, and the fly emerges from its pupa shell. The process of emergence is a relatively quick one. The fly breaks through the portion of the pupa that used to be the head section, crawls out, dries, then spreads its wings and flies away in less than five minutes (Dortmans *et al.,* 2021).

The fly lives for about a week after it emerges. It will look for a partner, copulate, and (for the female) deposit eggs throughout its brief existence. BSF does not feed and keep hydrated; they need a water supply or a humid surface. In this period of life, plenty of natural light and a warm temperature (25-32°C) is essential. A humid environment may lengthen life expectancy and, as a result, increase the likelihood of successful reproduction. The flies have been reported to prefer to copulate in the dawn light. Following copulation, the females look for a suitable spot to deposit their eggs (Dortmans *et al.,* 2021).

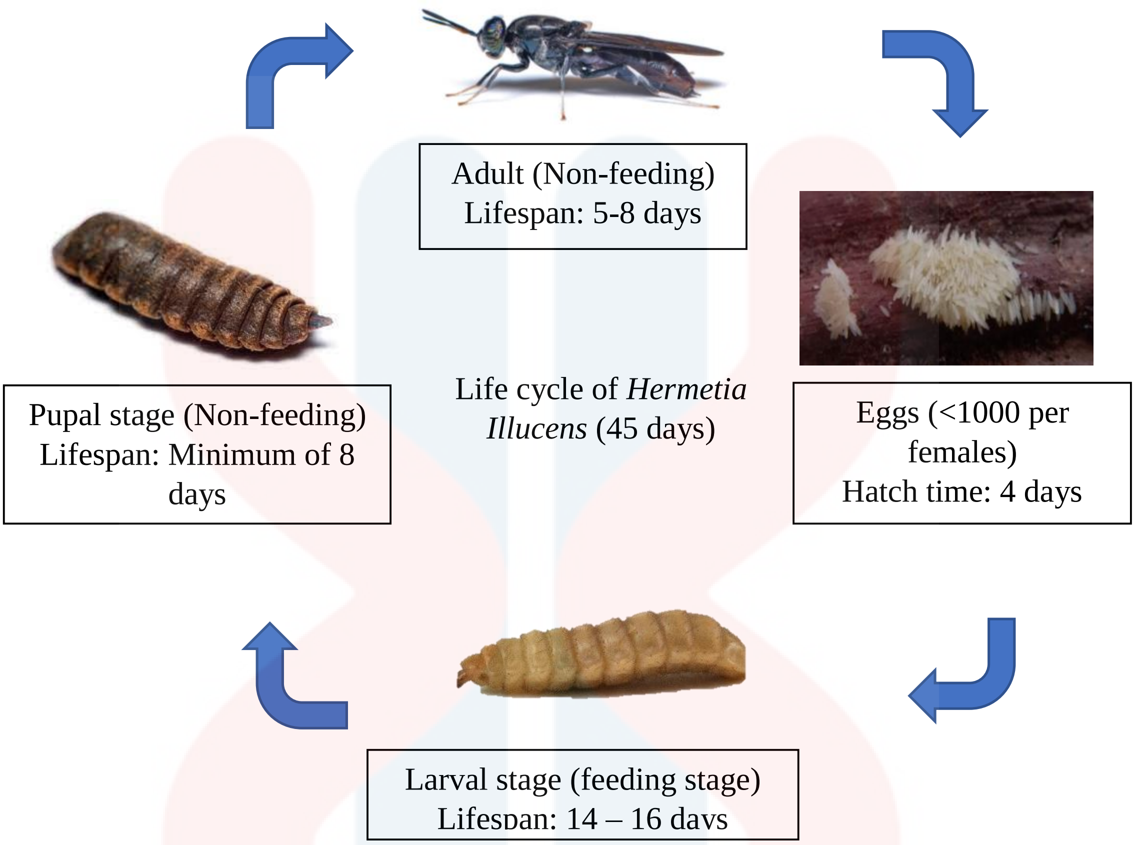

Figure 1. Life Cycle of Black Soldier Fly (*Hermetia Illucens*)

6.2 Nutritional values of Black Soldier Fly

The body composition of the Black Soldier Fly larvae varies between substrates not only in terms of protein content (which ranges from 37 to 63% dry matter, DM) but also in terms of fat content, which varies the most (ranging from 7 to 39% DM) (Barragan-Fonseca *et al.*, 2017). Even though BSF larvae have a high average amount of protein and fat (St-Hilaire *et al.*, 2007; Zheng *et al.*, 2012), their body composition depends on the quality and amount of food they eat (Newton *et al.*, 2005; Gobbi, 2012; Nguyen *et al.*, 2012). For example, larvae fed swine manure have more protein than larvae fed cow manure (Newton *et al.*, 2005; St-Hilaire *et al.*, 2007), while larvae fed spent grain has more protein (Oonincx *et al.*, 2015). The same can be said of crude fat. Black Soldier Fly larval biomass fed on manures had a fat content of roughly 30%, although chicken manure promoted maximum larvae development and crude fat content (Li *et al*., 2011). Different larval ages affect the BSFL nutrient composition and its bioavailability for animal feeds (Lie *et al.*, 2017). Therefore, it is necessary to evaluate the nutritional composition of various ages of BSFL for animal feed. For example, crude protein content declines with age, with the highest percentage observed for larvae aged 5 days (61%), and lower percentages reported for larvae aged 15 (44%), and 20 (42%), days (Rachmawati *et al.*, 2010). Fresh larvae have a dry matter content of 20 to 44 % (Sheppard *et al.*, 2008; Diener *et al.*, 2009; Nguyen *et al.*, 2015; Oonincx *et al.*, 2015), which varies depending on food and stage of larvae (Rachmawati *et al.*, 2010).

6.3 Growth performance of Black Solider Fly

In substrates with 45–75 % moisture content, BSF larvae thrived and matured into prepupae, reaching 299–403 mg wet weight (Bekker *et al.*, 2021). Depending on the substrate, prepupae typically weights 55–299 mg WW, but weights as high as 373 mg have been seen (Lalander *et al.*, 2020). Maximum weights were obtained on days 13–19, with the driest substrates reaching them first. Wet substrates allowed BSF larvae to grow longer than dry substrates (Fatchurochim *et al.*, 1989). The larvae grew larger at 65 % and 75 % substrate moisture levels than at 45 % and 55 % substrate moisture content. In addition, the maximum specific growth rate was 45 % compared to 75 % substrate moisture content. Before, maximum specific growth rates on different substrates were found by fitting sigmoidal curves to larval body widths (Sripontan *et al.*, 2020). These values fall within a wide range of those rates. Specific growth rates calculated from the weights of the larvae (Bekker *et al.*, 2021) found that the highest value was at the lowest level of moisture in the substrate.

7.0 MATERIALS AND METHOD

7.1 Study design of intensive farming method and production of Black Soldier Fly larvae

An experiment of intensive farming method of Black Soldier Fly Larvae was carried out at Teaching Farm, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, 16310 Bachok Kelantan.

A mating cage (Appendix A 2) was constructed out of reclaimed furniture wood and plastic nets as netted enclosures for a cohort of flies that emerged from the pupation container. The flies were given water to drink in the cage, a banana trunk as a shelter, and egg sticks to lay their eggs and an attractant container. The drinking water is available *ad libitum*. The cage faced south so flies could mate more quickly and get as much sunlight as possible. Mating was more likely occurred when the condition have adeqaute natural light, warm, and high of moisture in the air. A bulb was set up at the upper part of the cage and turned on during the night to stimulate the mating process.

Attractant containers (Appendix A 5) filled with substances drew the flies to lay their eggs on the egg sticks. Egg sticks were placed at the upper part of the container, and a net filtration was placed between the egg sticks and the container to prevent flies from entering the attractant container. Effective microbes, fermented fruit, dead flies, and bedding residue were the main attractants for the flies in this study. Black Soldier Fly eggs have also been discovered to attract other BSFs. Because of this, it was best not to collect eggs every day since the eggs left behind would make more females want to lay eggs.

A nursery container (Appendix A 3) was placed for the eggs to hatch. The eggs deposited between the egg sticks above the attractant container were transferred to the nursery container. Before being transferred into the nursery container, the eggs were weighed with a precision balance. Then, the harvested eggs hatched within 2-5 days. In the nursery container, five-day-old larvae were fed protein meal and fruit waste until they transformed into prepupae. On average, the eggs hatch in four days, and the newly hatched larvae will look for food and eat organic trash nearby.

A transfer container (Appendix A 4) was a system to collect the prepupae that crawled out of the nursery container to be ready for the pupation stage. The nursery container was placed inside the transfer container after two and a half weeks of larval development. The transfer container produced darkness, which helped the prepupae crawl out of the nursery container, searching for dry places to pupate. The transfer container's characteristic was dry, preventing the prepupae from escaping. Prepupae crawling into the transfer container was collected and moved to a pupation container.

The larvae turned into prepupae in the nursery container by approximately two and a half weeks and were moved to the transfer container. They undergo the pupation stage in the pupation containers (Appendix A 6) filled with rice husk to retain the adult population they can bury and where the prepupae pupate. The rice husk produced a dark environment, which helped facilitate the pupation process. Then, the pupation container is transferred into the mating cage and eventually emerges as fresh adult flies.

7.2 Evaluation of growth performance of Black Soldier Fly Larvae

An experiment with three groups of BSFL was carried out at Teaching Farm, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, 16310 Bachok Kelantan.

The treatment groups were divided into three different feed substrate groups: (A) chicken manure with fruit wastes served as a control group, (B) Bokashi protein meal with fruit wastes, and (C) soybean meal with fruit waste as the experimental groups. For each feeding substrate group, 1g of 5-day-old BSF eggs were placed, and the growth performances were assessed for 20 days. The data were recorded every three days until achieving two and a half weeks of age. The equipment for data collection was a measuring ruler and a weighing scale. The parameters (Appendix) were focused on weight (g), length (cm), height (cm), and width (cm). Random samples were used for each group as an average parameter during data collection.

7.3 Data Analysis of growth performance of Black Soldier Fly Larvae

In this study, a one-way ANOVA test was used to look at the data to figure out the type of substrates given and how well Black Soldier Fly larvae grew. The test was performed using SPSS Version 27 software.

7.4 Evaluation of Nutritional Composition in Black Soldier Fly Larvae

The nutritional composition of BSFL was evaluated after it had been partially dried using the sand roasting method (Appendix B 1). Group B had the highest growth rate compared to Groups A and C. Group B was chosen to assess the nutritional composition by proximate analysis at UNIPEQ, Universiti Kebangsaan Malaysia. Representatives of dried BSFL were analysed for total ash (Method: 923.03), moisture (Method: 950.46), total fat (Method: 991.36), crude protein (Method: 981.10), crude fibre (Method: 962.09) (AOAC 20th Edition, 2016). While carbohydrate and energy were analysed by Promerance Food Analysis: Theory and Practice, 2nd Ed. (page 637) and Pearson's The Chemical Analysis of Foods (6th Edition, page 578), respectively.

8.0 RESULTS

8.1 Evaluation of growth performance of Black Soldier Fly Larvae

The growth performance of BSFL was assessed over 20 days. For each feeding substrate group, 1 g of 5-day-old BSF eggs were placed, and the data were recorded every three days until the eggs reached two and a half weeks of age. Each group was given an equal amount of feed during the study period. For example, after 20 days, the BSFL in Group A had eaten 2.7 kg of chicken manure with rice husk, 4 kg of fruit waste (including mango, watermelon, guava, grape, orange, pineapple, and dragon fruit), and 1 L of effective microbes. For Group B, the BSFL has consumed 2.7 kg of Bukashi protein meal, 4 kg of fruit waste, and 1 L of effective microbe. Group C BSFL ate 2.7 kg of soybean meal, 4 kg of fruit waste, and 1 L of effective microbes. Parameters (Appendix C) such as weight, length, height, and width were taken from each group during the study period to study their growth performance on different feeding substrates. During data collection, each group used a random sample as a mean.

Table 1. Group A (Chicken manure with fruit wastes)

Parameters/ Day Old Larvae (DOL)	Weight (g)	Length (cm)	Height (cm)	Width (cm)
1-DOL	1g/container	-	-	-
4-DOL	0.0012g/larvae	4	1	1
7-DOL	0.013g/larvae	9.5	2	3
10-DOL	0.1g/larvae	15	3	4
14-DOL	0.15g/larvae	18.5	4	5
17-DOL	0.2g/larvae	22	5	6
20-DOL	0.2g/larvae	23	5	6

Table 2. Group B (Bukashi protein meal with fruit wastes)

Parameters/ Day Old Larvae (DOL)	Weight (g)	Length (cm)	Height (cm)	Width (cm)
1-DOL	1g/container	-	-	-
4-DOL	0.004g/larvae	9	2	2
7-DOL	0.033g/larvae	13	3	4
10-DOL	0.15g/larvae	18	4	5
14-DOL	0.2g/larvae	19	4	5
17-DOL	0.2g/larvae	22	5	6
20-DOL	0.2g/larvae	23	6	6.5

Table 3. Group C (Soybean meal with fruit wastes)

Parameters/ Day Old Larvae (DOL)	Weight (g)	Length (cm)	Height (cm)	Width (cm)
1-DOL	1g/container	-	-	-
4-DOL	0.0019g/larvae	5	1	1
7-DOL	0.007g/larvae	9	2	3
10-DOL	0.1g/larvae	14	3	4
14-DOL	0.15g/larvae	18	4	4.5
17-DOL	0.2g/larvae	22	4	5
20-DOL	0.2g/larvae	22	5	5

Based on the tables above, the results showed Group B, fed with Bukashi protein meal and fruit waste, has the highest growth performance compared to Group A and Group C. Each group achieved an average of 0.2 g in the final body weight of larvae. Groups A and B showed the most extended length of larvae, which is 23 cm on average. For the height of the larvae, Group B showed the highest height compared to other groups, which was 6 cm. Group B also showing the most extended width parameter of the larvae, which was 6.5 cm.

Figure 2 shows the data on BSFL weight after 20 days. The horizontal x-axis represents day-old larvae (DOL) for 20 days, while the vertical y-axis represents the weight of BSF larvae in grams. The line graph has an increasing slope, which means Groups A, B, and C showed excellent results in terms of growth performance. However, Group B was the fastest to achieve the maximum weight (0.2 g).

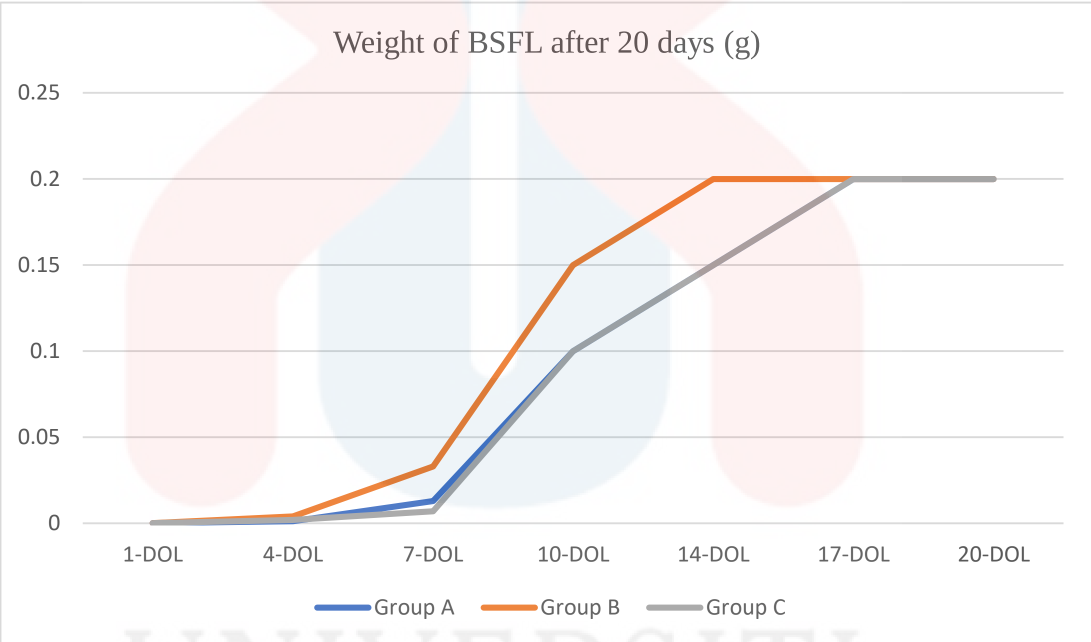

Figure 2. Weight of BSFL after 20 days

Figure 3 provides the data on BSFL length after 20 days. The horizontal x-axis represents day-old larvae (DOL) over 20 days, while the vertical y-axis represents the length of BSF larvae in cm. The line graph has an increasing slope, meaning Groups A, B, and C showed great growth results in terms of length parameters. But Groups A and B got the most extended length, 23 cm, compared to the Group C.

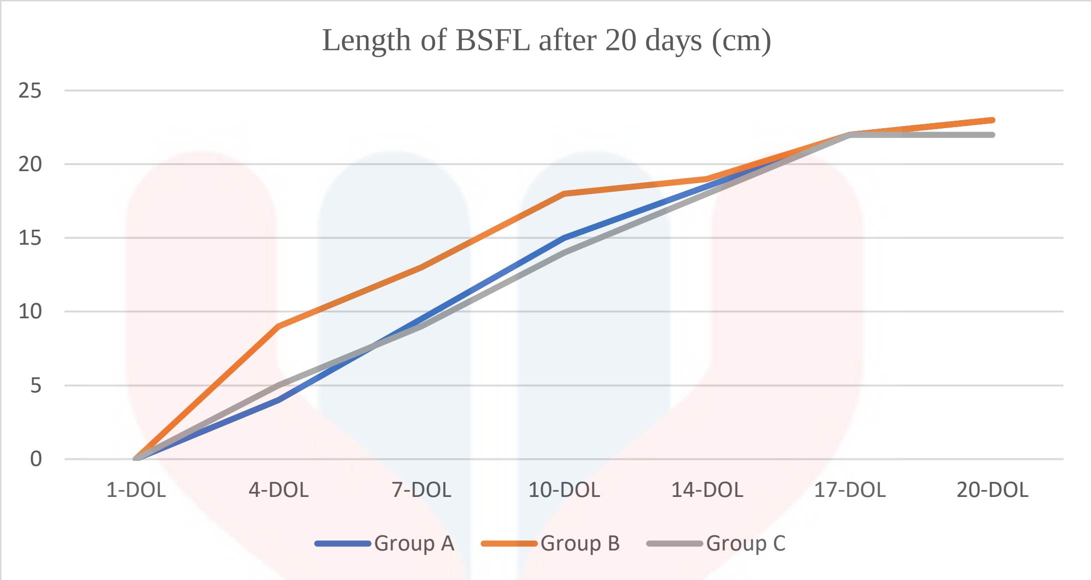

Figure 3. Length of BSFL after 20 days

Figure 4 provides the data on BSFL height after 20 days. The horizontal x-axis represents day-old larvae (DOL) over 20 days, while the vertical y-axis represents the height of BSF larvae in cm. The line graph has an increasing slope, meaning Groups A, B, and C showed positive results in terms of height parameters. But Group B had the highest height, 6 cm, compared to the other groups.

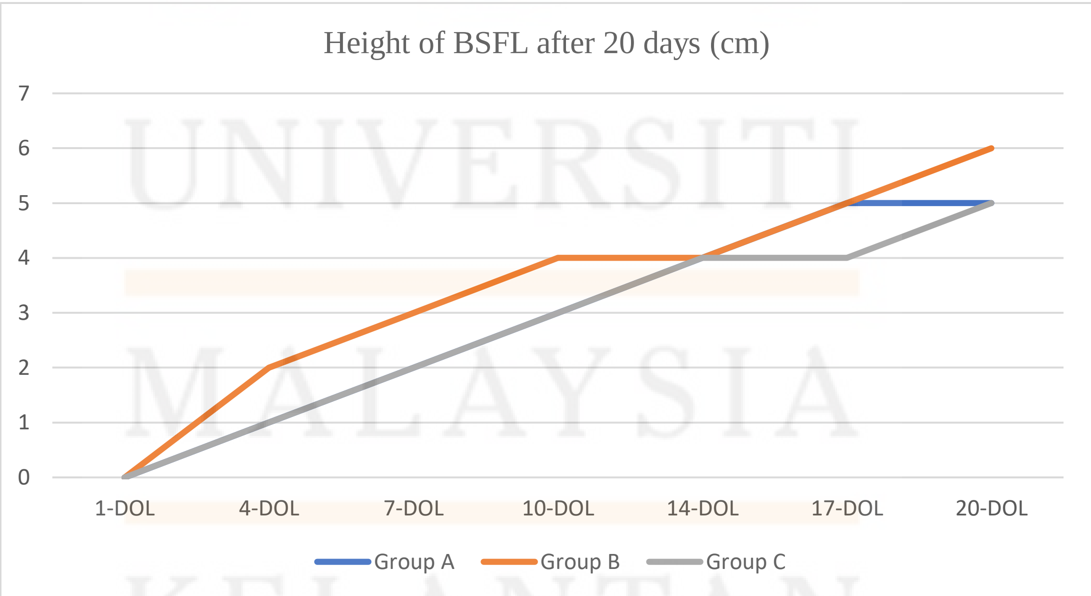

Figure 4. Height of BSFL after 20 days

Finally, Figure 5 shows the data on BSFL width after 20 days. Again, the horizontal x-axis represents day-old larvae (DOL) over 20 days, while the vertical y-axis represents the width of BSF larvae in cm. Similarly, the line graph has a slope that goes up, meaning Groups A, B, and C all show significant growth in width. But Group B had the widest width, 6.5 cm, compared to the other groups.

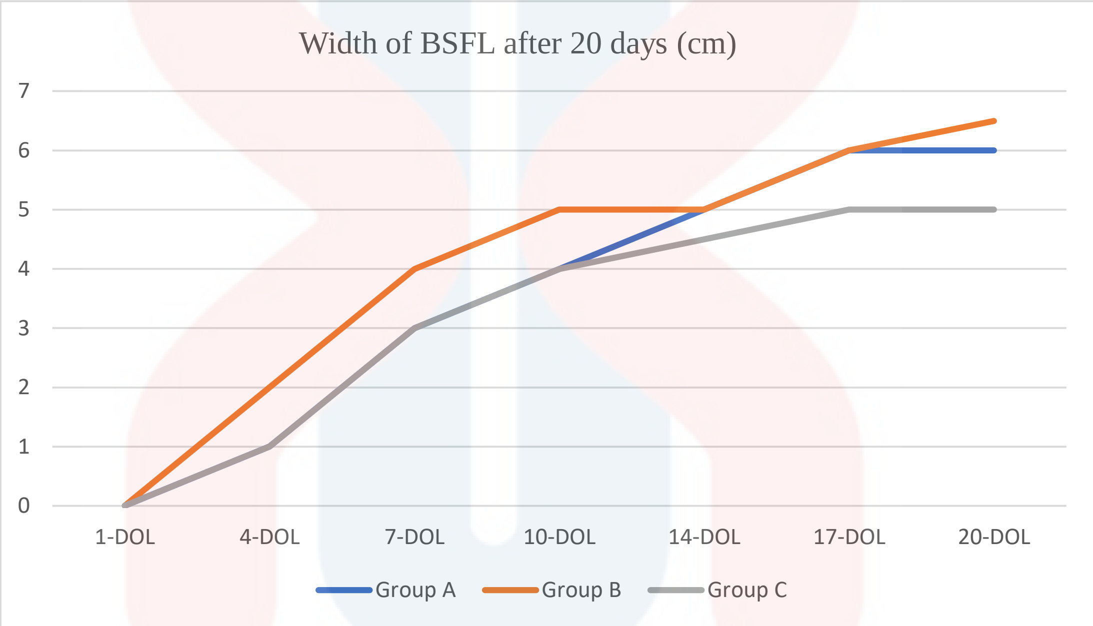

Figure 5. Width of BSFL after 20 days

8.2 Analysis of growth performance of Black Soldier Fly Larvae

The null hypothesis for this study is to know that the feeding substrate does not affect the BSFL growth performance while alternate hypothesis is that the feeding substrate does affect the growth performance of BSFL. The general rule of thumb for this test is that if the *p*-value (sig.) is less than 0.05, it will reject the null hypothesis. Conversely, if the *p*-value exceeds 0.05, it will accept the null hypothesis.

Table 4. one-way ANOVA using SPSS Version 27 Software

		Sum of Squares	df	Mean Square	F	Sig.
Weight	Between Groups	.002	2	.001	.09	.92
	Within Groups	.155	18	.009		
	Total	.157	20			
Length	Between Groups	16.381	2	8.190	.11	.90
	Within Groups	1317.071	18	73.171		
	Total	1333.452	20			
Height	Between Groups	2	2	1	.27	.76
	Within Groups	66	18	3.667		
	Total	68	20			
Width	Between Groups	2.595	2	1.298	.26	.77
	Within Groups	89.857	18	4.992		
	Total	92.452	20			

Remarks:

1. df: Degree of freedom
2. Sig: *p*-value (0.05)

From the table above, the results of the one-way ANOVA showed the larvae's average weight, length, height, and width were unaffected ($p > 0.05$) by the various feeding substrates provided. So, the analysis accepted the null hypothesis, which could explain why the p-value was higher than 0.05.

8.3 Evaluation of Nutritional Composition in Black Soldier Fly Larvae

Evaluating the nutritional contents of BSFL was done after partially drying the larvae using the sand roasting method. Group B was chosen to assess the nutritional value because it shows the highest growth performance compared to Groups A and C. The dried larvae were sent to UNIPEQ, UKM, Bangi, and the nutritional composition was assessed by proximate analysis.

Table 5. Proximate analysis of Group B

Test Description	Unit	Result(s)	Test method/ Equipment/Technique
Total Ash	g/100g	8.8	In-house Method No: STP/Chem/A05 based on AOAC 20th Edition: 923.03
Moisture	g/100g	34.1	In-house Method No: STP/Chem/A04 based on AOAC 20th Edition: 950.46
Total Fat – Soxhlet	g/100g	25.5	In-house Method No: STP/Chem/A02 based on AOAC 20th Edition: 991.36
Protein	g/100g	21.0	In-house Method No: STP/Chem/A03 based on AOAC 20th Edition: 981.10
Carbohydrate (by difference)	g/100g	10.6	In-house Method No: STP/Chem/A06 based on Promerance Food Analysis: Theory and

			Practice, 2nd Ed. (pg 637)
Energy (by calculation)	kcal/100g	356	In-house Method No: STP/Chem/A01 based on Pearson's The Chemical Analysis of Foods (6th Edition, page 578)
Crude fibre	g/100g	10.2	In-house Method, Ref. No: STP/Chem/A08 based on AOAC 20th Edition, 962.09

Remarks:

1. %Total Carbohydrate = 100 – (%Ash + %Moisture + %Protein + %Fat)
2. Protein factor: 6.25

The table above shows the result of the proximate analysis of group B. According to the results, the crude protein is 21.0 g/100g, while the total fat is 25.5 g/100g. The moisture content at 34.1 g/100g, the weight of the crude fiber is 10.2 g/100g, while the total ash is 8.8 g/100g. Carbohydrates have a difference of 10.6 g/100g.

9.0 DISCUSSION

The intensive farming approach in the production of BSFL was selected in this research due to some factors. For smallholder farmers, it is a more convenient method in terms of cost and management. Other than the low cost of equipment and materials, the feed substrates/waste products could be found easily in nearby supermarkets, abandoned construction sites, fruit stands, small poultry farms, and others. The intensive farming method also requires low maintenance and little labour. Even though extensive farming is considered cheap, it takes longer to get wild adult flies to breed, and it is hard to keep track of the group of flies. This is because the prevalence of Black Soldier Flies in Malaysia is lower than that of blowflies (*Calliphoridae*), houseflies (*Musca domestica*), and others. Besides, it is hard to attract wild female flies to lay their eggs in the attractant container full of effective microbes, fermented fruit, dead flies, and bedding residue. To get around these issues, purchasing BSF eggs from an established farm company that engages in large-scale intensive BSF cultivation could be an alternative to starting up the intensive farming of BSF.

Besides, a few issues were observed in establishing intensive BSF farming in this study. A mosquito net served as a mating cage once the fly matured. The larvae survived the first cycle and emerged as flies in the mating cage, but it has been shown that pests like ants, wasps, and birds can get inside the net and eat the larvae. After that, the mating cage was re-invented using wood from old furniture and plastic nets. In order to manage the pests, four ant traps were placed at the cage sides in each mating cell, while water and oil were placed into the container to stop the ant from climbing the cage. Then, some areas of the cage were not receiving enough light, despite the cell being oriented south to maximize the quantity of sunlight, which is essential for successful copulation among adult flies. As an alternative method, an LED bulb was installed above the cage to provide artificial heat and light at night, which also helps the flies copulate.

The results of the growth performance of BSF larvae fed with different substrates demonstrated Group B, which was given Bukashi protein meal and fruit waste, had the highest growth performance. The final body weight of the larvae was averaged out across all groups at 0.2 g, and Group B had a higher final body weight as compared to Groups A and C. Larvae from Groups A and B were the longest, with an average length of 23 cm. Group B's larvae had a height of 6 cm compared to the other groups, which was the highest. Additionally, Group B displayed the larvae with the broadest width parameter, measuring 6.5 cm. Based on observation, these results may be attributable to the Bukashi protein meal, which attracts BSF larvae to feed more than other groups when provided with sufficient moisture and scents. As shown by group C, in which the larvae were provided with soybean meal and fruit waste, the colony of larvae had difficulties when the substrate lack of airflow. According to Yang (2017) and Zhang (2021), poor substrate ventilation could cause the larvae to stop feeding and release heat at the top of the substrate. The larvae tend to crawl out of the container, creating a favourite growing spot.

Additionally, inadequate humidity and excessive moisture can suffocate and kill larvae. Excessive substrate moisture could cause the substrate to sink to the bottom of the container and prevent the larvae from crawling out to the container surface, which may lead to the death of the larvae due to lack of oxygen or undissipated excessive heat (Yang, 2017). High temperatures are another thing that could kill the larvae colony, as shown by groups B and C, where the larvae gathered on top of the substrate to get rid of heat and moved outside the container to find a cooler place. According to Evans (2016) the larvae usually stop feeding once the temperature reaches above 44 degrees Celcius. Suppose the temperature is above 44 degrees Celcius for over 4 hours. In that case, it will cause the larvae to eventually die, which could lead to significant damage in the colony and affect the growth performance of the BSFL.

Besides, this study revealed that the average weight of the larvae was not influenced ($p > 0.05$) by the varied feeding substrates offered based on the growth performance of BSFL. Nevertheless, Group B displayed the fastest average final weight of larval development at 0.2 g compared to Groups A and C. The period taken to reach 0.2 g in Group B was just 14 days, compared to Groups A and C, which took 17 days. This could be because each feeding substrate has a different moisture content. According to Sripontan *et al*. (2020), the larvae grow larger at 65 % to 75 % substrate moisture levels than at 45 % to 55 % substrate moisture content. Depending on the type of feeding substrates given to the larvae, which also had an impact on the weight of the larvae, the moisture level of the BSFL body content might range from 60 to 80 % per larva. So, by introducing more fruit waste, effective microbes, or tap water, the moisture content might be raised, thus increasing the weight of the larvae.

Additionally, this outcome might result from the larvae already reaching their average maximum weight or the less sensitive weighing equipment used in this experiment. Based on the BSFL's growth performance in this research, the larvae's average length, height, and width were also unaffected ($p > 0.05$) by the various feeding substrates provided. Compared to Groups A and C, Group B displayed the expanded final length, height, and width of the BSFL with measurements of 23 cm, 6 cm, and 6.5 cm, respectively. The feeding substrate in Group B had a higher proportion of crude protein and crude fat, which allowed the larvae to grow longer and wider. This could account for the higher values observed. Also, Group B may have grown better than the other groups because it had high moisture. Because the variations between the parameters were so minor and the mean value differences between the groups were so minimal, the analysis accepted the null hypothesis, which could explain why the *p*-value was higher than 0.05.

The nutritional composition of feeding substrates from Group B was tested because it revealed the highest growth performance of the BSF larvae as compared to other groups. The findings show a crude protein of 21.0 g/100 g, total fat of 25.5 g/100 g, and a difference of 10.6 g/100 g for carbohydrates. The moisture content was 34.1 g/100 g, 10.2 g/100 g of the total weight was crude fiber, and 8.8 g/100 g was total ash. In comparison to other studies that obtained crude protein and fat percentages of up to 30 and 50%, respectively, these percentages were deemed low. 53.86% crude protein, 7.74% ash, 33.73% crude fat, and 7.82% moisture were the nutritional composition of BSF larvae (Wirat *et al*., 2021) in research that was carried out in Thailand. For crude protein, crude fiber, crude fat, and ash, respectively, the nutrient composition of BSF larvae raised with mixed organic wastes ranges in Indonesia from 42% to 47%, 7% to 9%, 11.8% to 34.8%, and 14.6% to 15.9% (Astuti & Wiryawan, 2022).

The results of nutritional composition observed in this study were contributed by less concentrated dry matter. It is shown that drier larvae have a higher protein and fat content than partially dried larvae since the moisture in a larva evaporates, concentrating the remaining nutrients in the product (Baraggan *et al*., 2017). Besides, fresh larvae are highly vulnerable to lipid oxidation, enzymatic degradation, and microbiological deterioration because of the high-water activity (Rahman, 2007). Thus, removing and limiting water activity makes the substance more storable and suppresses microbial and enzymatic activity. Dried larvae have more protein than fresh larvae because the product's remaining nutrients are concentrated due to water evaporation. The roasting process should be longer, with enough heat to dry the larvae properly and get the most protein out. Different drying techniques for the larvae could be considered, such as heating in an oven, using a microwave, or sand roasting in a drum.

10.0 CONCLUSION

In conclusion, the intensive farming method of Black Soldier Fly is recommended in Malaysia compared to the extensive farming method. The growth performance of feeding the BSF larvae with chicken manure, Bokashi protein meal, and soybean meal showed similar results. Thus, the growth performance of BSFL is unaffected by the various feeding substrates. The nutritional analysis showed that the crude protein and crude fat were regarded as low for this research due to the improper drying method of the BSF larvae.

11.0 RECOMMENDATIONS AND FUTURE WORK

Several limitations were observed during this project. For future studies, a complete nutritional analysis should be conducted to compare the nutrient composition, especially protein level, between the groups with different feeding substrates. Comparison between alternative protein supplements, such as maise and fishmeal, should also be conducted to know the advantages and limitations of each supplementation.

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REFERENCES

1. Abd El-Hack, M., Shafi, M., Alghamdi, W., Abdelnour, S., Shehata, A., Noreldin, A., Ashour, E., Swelum, A., Al-Sagan, A., Alkhateeb, M., Taha, A., Abdel-Moneim, A.-M., Tufarelli, V., & Ragni, M. (2020). Black Soldier Fly (*Hermetia illucens*) Meal as a Promising Feed Ingredient for Poultry: A Comprehensive Review. Agriculture, 10(8), 339. https://doi.org/10.3390/agriculture10080339

2. Astuti, D. A., & Wiryawan, K. G. (2022). Black soldier fly as feed ingredient for ruminants. *Animal Bioscience*, *35*(2), 356–363. https://doi.org/10.5713/ab.21.0460

3. Barragan, K. B., Dicke, M., & Loon. (2017). Nutritional value of the black soldier fly (*Hermetia illucens*) and its suitability as animal feed. ResearchGate; Wageningen Academic Publishers. https://www.researchgate.net/publication/317221082_Nutritional_value_of_the_black_soldier_fly_Hermetia_illucens_L_and_its_suitability_as_animal_feed

4. Bram Dortmans, Diener, S., Egger, J., & Zurbrügg, C. (2021, August). Black Soldier Fly Biowaste Processing - A Step-by-Step Guide, 2nd Edition. ResearchGate; Eawag: Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland.

5. Čičková, H., Newton, G. L., Lacy, R. C., & Kozánek, M. (2015). The use of fly larvae for organic waste treatment. Waste Management, 35, 68–80. https://doi.org/10.1016/j.wasman.2014.09.026

6. Craig Sheppard, D., Larry Newton, G., Thompson, S. A., & Savage, S. (1994). A value added manure management system using the Black Soldier Fly.

Bioresource Technology, 50(3), 275–279. https://doi.org/10.1016/0960-8524(94)90102-3

7. Dennis, Sarah van Broekhoven, Arnold Van Huis, & Loon. (2015, December 24). Feed Conversion, Survival and Development, and Composition of Four Insect Species on Diets Composed of... ResearchGate; Public Library of Science. https://www.researchgate.net/publication/288001680_Feed_Conversion_Survival_and_Development_and_Composition_of_Four_Insect_Species_on_Diets_Composed_of_Food_By-Products
8. Diener, S., Zurbrügg, C., & Klement Tockner. (2009). Conversion of organic material by black soldier fly larvae: Establishing optimal feeding rates. ResearchGate; SAGE Publications. https://www.researchgate.net/publication/26272272_Conversion_of_organic_material_by_black_soldier_fly_larvae_Establishing_optimal_feeding_rates#:~:text=and%20kitchen%20waste).-,Diener%20et%20al.,promote%20sustainable%20agriculture.
9. Erickson, M. C., Islam, M., Sheppard, C., Liao, J., & Doyle, M. P. (2004). Reduction of Escherichia coli O157:H7 and Salmonella enterica Serovar Enteritidis in Chicken Manure by Larvae of the Black Soldier Fly. Journal of Food Protection, 67(4), 685–690. https://doi.org/10.4315/0362-028x-67.4.685
10. Evans Nyakeri, Joseph, H., Ayieko, M., & Fred Anangwe Amimo. (2016). an open system for farming Black Soldier Fly larvae as a source of proteins for smallscale poultry and... ResearchGate; Wageningen Academic Publishers. https://www.researchgate.net/publication/311528078_An_open_system_for_f

arming_black_soldier_fly_larvae_as_a_source_of_proteins_for_smallscale_poultry_and_fish_production

11. Evans Nyakeri, Joseph, H., Ayieko, M., & Fred Anangwe Amimo. (2017). Valorization of organic waste material: Growth performance of wild black soldier fly larvae (Hermetia... ResearchGate; Wageningen Academic Publishers. https://www.researchgate.net/publication/319606622_Valorisation_of_organic_waste_material_Growth_performance_of_wild_black_soldier_fly_larvae_Hermetia_illucens_reared_on_different_organic_wastes
12. Fatchurochim, S., Geden, C. J., & Axtell, R. C. (1989). Filth Fly (Diptera) Oviposition And Larval Development In Poultry Manure Of Various Moisture Levels. Journal of Entomological Science, 24(2), 224–231. https://doi.org/10.18474/0749-8004-24.2.224
13. *Intensive Black Soldier Fly Farming*. (2017). Symton® Black Soldier Fly. https://symtonbsf.com/blogs/blog/intensive-black-soldier-fly-farming
14. Kawasaki, K., Hashimoto, Y., Hori, A., Kawasaki, T., Hirayasu, H., Iwase, S., Hashizume, A., Ido, A., Miura, C., Miura, T., Nakamura, S., Seyama, T., Matsumoto, Y., Kasai, K., & Fujitani, Y. (2019). Evaluation of Black Soldier Fly (*Hermetia illucens*) Larvae and Pre-Pupae Raised on Household Organic Waste, as Potential Ingredients for Poultry Feed. Animals, 9(3), 98. https://doi.org/10.3390/ani9030098
15. Kim, W., Park, K., Bae, S., Lee, S., Choi, Y., Han, S., & Koh, Y. (2011). Biochemical characterization of digestive enzymes in the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). Journal of Asia-Pacific Entomology, 14(1), 11–14. https://doi.org/10.1016/j.aspen.2010.11.003

16. Lalander, C., Wiklicky, V., Ermolaev, E., & Vinnerås, B. (2020). Process efficiency and ventilation requirement in black soldier fly larvae composting of substrates with high water content. Science of the Total Environment, 729, 138968. https://doi.org/10.1016/j.scitotenv.2020.138968

17. Mapanao, R., Jiwyam, W., Nithikulworawong, N., & Weeplian, T. (2021). Effects of black soldier fly (*Hermatia illucens*) larvae as a fish meal replacement on growth performance, feed utilisation, morphological characters and carcass composition of Thai climbing perch (*Anabas testudineus*). Journal of Applied Aquaculture, 1-15. https://doi.org/10.1080/10454438.2021.1923609

18. Rachmawati Rachmawati, Damayanti Buchori, Hidayat, P., & Melta Rini Fahmi. (2015, September 28). Perkembangan dan Kandungan Nutrisi Larva *Hermetia illucens* (Linnaeus) (Diptera: *Stratiomyidae*) pada Bungkil Kelapa Sawit ResearchGate; Entomological Society of Indonesia/Perhimpunan Entomologi Indonesia. https://www.researchgate.net/publication/321967891_Perkembangan_dan_Kandungan_Nutrisi_Larva_Hermetia_illucens_Linnaeus_Diptera_Stratiomyidae_pada_Bungkil_Kelapa_Sawit

19. Sheppard, D. C., Tomberlin, J. K., Joyce, J. A., Kiser, B. C., & Sumner, S. M. (2002). Rearing Methods for the Black Soldier Fly (Diptera: *Stratiomyidae*): Table 1. Journal of Medical Entomology, 39(4), 695–698. https://doi.org/10.1603/0022-2585-39.4.695

20. Sheppard, D. C., Tomberlin, J. K., Joyce, J. A., Kiser, B. C., & Sumner, S. M. (2002). Rearing Methods for the Black Soldier Fly (Diptera: *Stratiomyidae*):

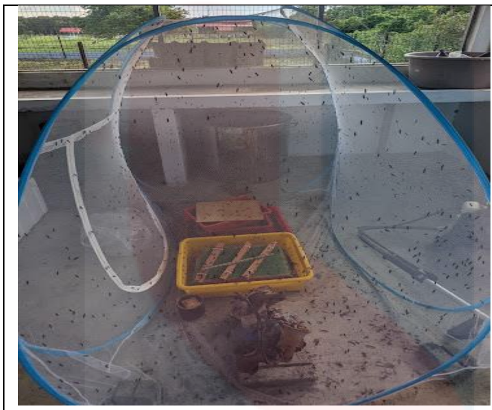
Table 1. Journal of Medical Entomology, 39(4), 695–698. https://doi.org/10.1603/0022-2585-39.4.695

21. Spranghers, T., Noyez, A., Schildermans, K., & De Clercq, P. (2017). Cold Hardiness of the Black Soldier Fly (Diptera: *Stratiomyidae*). Journal of Economic Entomology, 110(4), 1501–1507. https://doi.org/10.1093/jee/tox142
22. Sripontan, Y., Chiu, C.-I., Tanansathaporn, S., Leasen, K., & Manlong, K. (2019). Modeling the Growth of Black Soldier Fly *Hermetia illucens* (Diptera: *Stratiomyidae*): An Approach to Evaluate Diet Quality. Journal of Economic Entomology, 113(2), 742–751. https://doi.org/10.1093/jee/toz337
23. St-Hilaire, S., Cranfill, K., McGuire, M. A., Mosley, E. E., Tomberlin, J. K., Newton, L., Sealey, W., Sheppard, C., & Irving, S. (2007). Fish Offal Recycling by the Black Soldier Fly Produces a Foodstuff High in Omega-3 Fatty Acids. Journal of the World Aquaculture Society, 38(2), 309–313. https://doi.org/10.1111/j.1749-7345.2007.00101.x
24. Tomberlin, J. (2020). Biological, behavioral, and toxicological studies on the black soldier fly (Diptera: *stratiomyidae*). Undefined; https://www.semanticscholar.org/paper/Biological%2C-behavioral%2C-and-toxicological-studies-Tomberlin/57e2a11f6b85a8e87a9112932da91d6a22da5de7
25. Wang, Y.-S., & Shelomi, M. (2017). Review of Black Soldier Fly (*Hermetia illucens*) as Animal Feed and Human Food. Foods, 6(10), 91. https://doi.org/10.3390/foods6100091
26. Zhang, J., Zhang, J., LI, J., Tomerlin, J. K., Xiao, X., ur Rehman, K., Cai, M., Zheng, L., & Yu, Z. (2021). Black soldier fly: A new vista for livestock and

poultry manure management. Journal of Integrative Agriculture, 20(5), 1167–1179. https://doi.org/10.1016/s2095-3119(20)63423-2

APPENDICES

Appendix A: Intensive farming method of BSFL



A 1: Mosquito Net

A 2: Mating Cage

A 3: Nursery container

A 4: Transfer container

A 5: Adult BSF deposited its eggs between the eggs sticks above the attractant container

A 6: Pupation container

Appendix B: Sampling for nutritional analysis

B 1: Sand roasting method

B 2: Sand roasting method

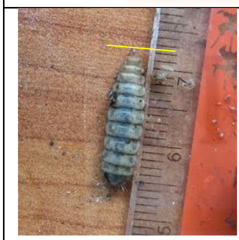
B 3: Vacuum the packaging to avoid any damage to the sample

B 4: weighed the total weight of the sample before sending to UKM, UNIPEQ for nutritional analysis

Appendix C: Growth performance of BSF larvae

 **DOL:** **1g/container**	 **4-DOL:** **0.0012g/larvae**	 **7-DOL:** **0.013g/larvae**	 **10-DOL:** **0.1g/larvae**
 **14-DOL:** **0.15g/larvae**	 **17-DOL:** **0.2g/larvae**	 **20-DOL:** **0.2g/larvae**	Remarks: Blue bottle cap: 1.2g Green bottle cap: 1.0g

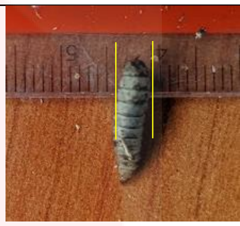
Appendix C 1a: Weight of BSFL in 20 days (Group A)

 4-DOL: 4cm	 7-DOL: 9.5 cm	 10-DOL: 15cm
 14-DOL: 18.5cm	 17-DOL: 22cm	 20-DOL: 23cm

Appendix C 1b: Length of BSFL in 20 days (Group A)

 4-DOL: 1cm	 7-DOL: 2cm	 10-DOL: 3cm
 14-DOL: 4cm	 17-DOL: 5cm	 20-DOL: 5cm

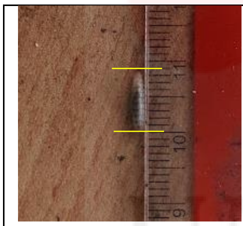
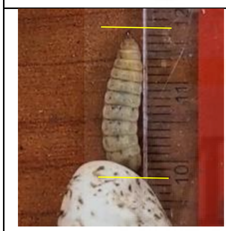
Appendix C 1c: Height of BSFL in 20 days (Group A)

 4-DOL: 1cm	 7-DOL: 3cm	 10-DOL: 4cm
 14-DOL: 5cm	 17-DOL: 6cm	 20-DOL: 6cm

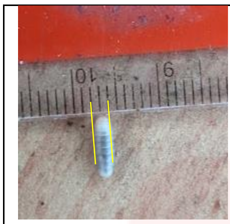
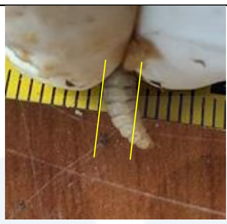
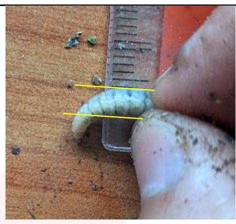
Appendix C 1d: Width of BSFL in 20 days (Group A)

 **DOL: 1g/container**	 **4-DOL: 0.004g/larvae**	 **7-DOL: 0.007g/larvae**	 **10-DOL: 0.15g/larvae**
 **14-DOL: 0.2g/larvae**	 **17-DOL: 0.2g/larvae**	 **20-DOL: 0.2g/larvae**	Remarks: Blue bottle cap: 1.2g Green bottle cap: 1.0g

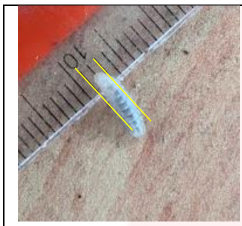
Appendix C 2a: Weight of BSFL in 20 days (Group B)

 4-DOL: 9cm	 7-DOL: 13cm	 10-DOL: 18cm
 14-DOL: 19cm	 17-DOL: 22cm	 20-DOL:23 cm

Appendix C 2b: Length of BSFL in 20 days (Group B)

 4-DOL: 2cm	 7-DOL: 3cm	 7-DOL: 4cm
 14-DOL- 4cm	 17-DOL- 5cm	 20-DOL: 6cm

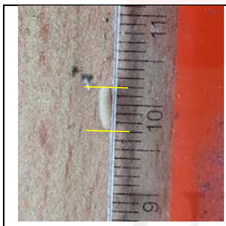
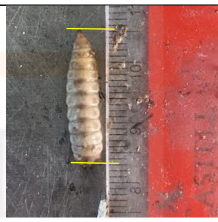
Appendix C 2c: Height of BSFL in 20 days (Group B)

 4-DOL: 2cm	 7-DOL: 4cm	 10-DOL: 5cm
 14-DOL: 5cm	 17-DOL: 6cm	 20-DOL: 6.5cm

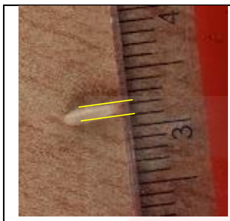
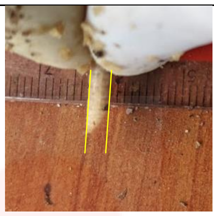
Appendix C 2d: Width of BSFL in 20 days (Group B)

 **DOL:** **1g/container**	 **4-DOL:** **0.0019g/larvae**	 **7-DOL:** **0.007g/larvae**	 **10-DOL:** **0.1g/larvae**
 **14-DOL:** **0.15g/larvae**	 **17-DOL:** **0.2g/larvae**	 **20-DOL:** **0.2g/larvae**	Remarks: Blue bottle cap: 1.2g Green bottle cap: 1.0g

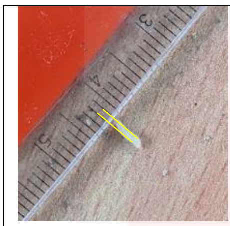
Appendix C 3a: Weight of BSFL in 20 days (Group C)

 4-DOL: 5cm	 7-DOL: 9cm	 10-DOL: 14cm
 14-DOL: 18cm	 17-DOL: 22cm	 20-DOL: 22cm

Appendix C 3b: Length of BSFL in 20 days (Group C)

 4-DOL: 1cm	 7-DOL: 2cm	 10-DOL: 3cm
 14-DOL: 4cm	 17-DOL: 4cm	 20-DOL: 5cm

Appendix C 3c: Height of BSFL in 20 days (Group C)

 4-DOL: 1cm	 7-DOL: 3cm	 10-DOL: 4cm
 14-DOL: 4.5cm	 17-DOL: 5cm	 20-DOL: 5cm

Appendix C 3d: Width of BSFL in 20 days (Group C)