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Research Article

Productivity and Nutritive Value of Black Soldier Fly (*Hermetia illucens)* Larvae Fed with Different Waste Substrates

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ABSTRACT

Due to the unavailability and rising market price of fishmeal and soybean meal, insect larvae, such as black soldier fly larvae (BSFL: Hermetia illucens), are currently being investigated as an alternate source of protein for swine and poultry. The productivity and nutritional value of BSFL were examined in this study together with the effects of various waste substrates.

There were two experimental containers for every treatment and were replicated three times and laid out using a Completely Randomized Design (CRD). A total of twenty-four (24) plastic containers (12 x 8.5 x 3 inches) were used which per container contained 100 grams/1000 of 4 days old BSF larvae.

The results showed that BSF larvae fed with T2-Fruit remains and T3 -Vegetable scraps yielded the highest production of 1230-1312 grams and an early maturation period of 18-19 days. Both T2-Fruit remains and T3 -Vegetable scraps have the highest nutritional value of around 43% to 47% of crude protein content, respectively as compared to the rest of the treatments. The nutritional profile of BSF larvae fed fruit and vegetable waste suggests that it may be used as a less expensive and more sustainable source of protein for pigs and poultry.

Keywords*: black soldier fly, larvae fed, nutritive value, productivity, waste substrates*

Introduction

Feed is the largest single cost item for livestock and poultry production, accounting for 60%–70% of the total cost in most years. Increasing demand for fishmeal and soybean meal as major protein sources in animal feeds has led to their scarcity and increasing market prices. The idea to use insects as a protein source in pig (and poultry) feed is embraced by an increasing number of specialists. Insects can be a sustainable feed ingredient in pig and poultry diets. Black soldier fly (Hermetica

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illucens) is one of the most promising for large-scale production. By using organic waste substances as substrates, the system is expected to contribute to considerable reduction of production costs.

The study aims to package technology of the Production of Black Soldier Fly Larvae Fed with recommended waste substrates as potential alternative protein source for swine and poultry. Specifically:

1. To determine the larva production performance fed with different waste substrates
2. To identify organic waste substrates suitable for the production of black soldier fly larvae.
3. To determine the reduction rate of the substrates given to BSF larvae
4. To determine the nutrient content of BSF larvae Meal fed with different waste substrates compared to the most commonly used protein sourced for animal feed like soybean oil meal and fish meal.
5. To determine the production cost of rearing Black Soldier Fly Larvae fed with different waste substrates.

Description and Life Cycle of H. illucens

H. illucens Linnaeus 1758 is a fly (Diptera) of the Stratiomyidae family. Adult fly is black, wasp-like and 15-20 mm long (Hardouin et al., 2003). Larvae can reach 27 mm in length, 6 mm in width and weigh up to 220 mg.

Environmental Impact

Adult flies are not attracted to human habitats or foods and are not considered a nuisance (Van Huis et al., 2013).

Housefly Control

Housefly larvae (Musca domestica) compete with black soldier fly larvae, which have been proven to lower the housefly population of pig or poultry dung by 94% to 100%. They can therefore aid in the reduction of housefly populations in households and livestock farms that practice inadequate hygiene.

Low Pathogenicity

The H. Illucens larvae alter the microbiota of manure, potentially lowering dangerous bacteria like Escherichia coli 0157:H7 and Salmonella enterica. Illucens is not a disease vector. There is a theory that the larvae contain organic antibiotics.

Fermented Rice bran for BSF larvae

Fermented rice bran can prolong the storage time of food for flies up to nearly 6 months. Fermented rice brandish is a kind of pickle for people to eat. Any food that you have finished mixing will not spoil if kept in a cool, shady place with a moderate temperature.

Methods

Experimental Design & Treatment

The study was laid out using a Completely Randomized Design (CRD). There were two experimental containers for every treatment and were replicated three times. A total of twenty-four (24) plastic containers (12 x 8.5 x 3) inches were used which per container contained 1 week old 100 grams or1000 larvae (hand-counted). The treatments were as follows:

T1 – Fermented rice bran (Control)
T2 - Fruit remains
T3 - Vegetable scraps
T4 - Swine manure

Time & Place of the Study

The study was conducted on March 1, 2020-August 2020 in Dumalinao Swine Breeding Center (DSBC), Paradise, Sumadat, Dumalinao, ZDS.

Source of Black Soldier Fly Larvae

The BSF larvae were sourced out from the BSF nursery maintained at Dumalinao Swine Breeding Center (DSBC), Paradise, Dumalinao, ZDS. The initial population was originally collected in the surroundings from the spoiled mixed feed and in the composting area with decaying fruit and vegetable waste. Since then we did some research on how to reproduce the BSF larvae and was maintained by the station for 6 months before use in this study.

Two Thousand Four Hundred (2400) grams of BSF larvae were used in the study per cycle placed in the 24 plastic containers. Each container contained 4 days old BSF larvae weighing 100 grams or 1000 larvae (hand-

counted). The young larvae used in the experiment were sieved with a mesh diameter of 1.2 mm, and only those that made it through the sieve on their own were employed. This was done to guarantee that the larvae were of a uniform size and mass.

Source of Waste Substrates

Fruit and vegetable scraps were collected from the Dumalinao Public Market and stored for 2 days to reduce the water content while swine manure was collected from the breeder sow maintained in the station and air-dried for 2 days.

Diets of BSF Larvae

Treatment	3 days old	4 days old until pre-pupae
1	Fermented Mixed Feed (100 grams)w/40 % moisture content	Fermented rice bran
2	Fermented Mixed Feed (100 grams) w/40 % moisture content	Fruit Remains
3	Fermented Mixed Feed(100 grams) w/40 % moisture content	Vegetable Scraps
4	Fermented Mixed Feed (100 grams) w/40 % moisture content	Swine Manure

The composition of fermented mixed feed for 7 days old BSF larvae was 2 kg rice bran, 1 kg hammered corn, ½ kg copra meal, ¼ kg molasses, and 1 liter water. The feed ingredients were mixed and fermented in empty margarine tab for 7 days.

Fermented rice bran (control) was composed of 1 kg of rice bran, 1/2 kg sweet potato leaves, 50 grams molasses, and 500 ml of water. After ingredients were mixed it was fermented in an empty margarine tab in 10 days before used in the study.

For the 3 days old of BSF Larvae they had been given 100 grams fermented mixed feed to make certain optimum larvae development whereas the 4 days old BSF larvae (1st instar) were given 250 grams of waste substrates per container based on their corresponding treatments and were replenished with 250 grams once a week as needed until more than 50% of larvae metamorphosed into prepupal.

Harvesting of Black Soldier Fly Maggots

Most of the larvae migrated from fattening plastic boxes to harvesting containers during their sixth instar. The larvae that were not migrated to the container were harvested using a sieving process (2 mm mesh diameter) to separate larvae and substrate residues.

Weight Increment of BSF Larvae

For all treatments, weighing was done on every 3rd day by randomly counting 50 larvae from each container. The total weight obtained was divided by the number of larvae to obtain an average weight of each larva. The final prepupa weight was taken to be the average weight recorded on the day of harvest.

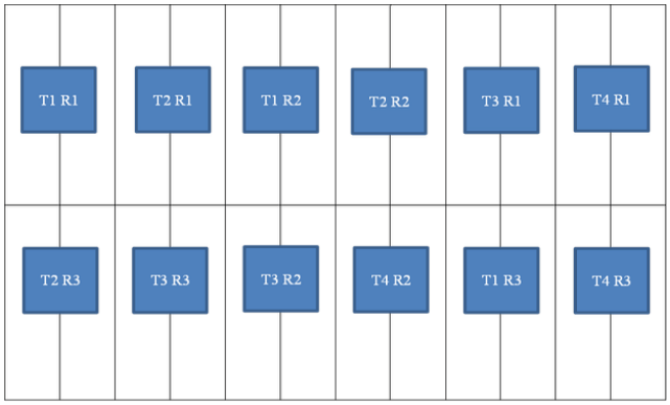
Nutrient Analysis of BSF Larvae

A sample of 250 g harvested prepupa was taken from each treatment were blanched in hot water for 5 minutes and then sun dried for 3 days. After drying, the samples were properly packed and sent at Regional Animal Feed Analysis Laboratory (RAFAL), Zamboanga City.

Data Analysis

One-way ANOVA ($P \leq 0.05$) with subsequent Least Significant Difference (LSD) tests were applied to detect differences in data collected among all treatments.

Experimental lay-out using Completely Randomized Design (CRD)



Legend:
T1- Fermented rice bran (Control)
T2- Fruit remains
T3- Vegetable Scraps
T4- Swine Manure

Process of Rearing Black Soldier Fly Maggots from 1st -4th Cycle Period

Preparation of BSF Culture

1. Wooden sheets with a small gap in between with eggs were used and placed in a container that will now serve as a BSF culture (Figure 1). An empty plastic container (L-7-inch x W-5-inch x H-2.5) was used.
2. The fermented feed (100 grams with 40% moisture content) inside the container was served as ready food for first instar larvae as they dropped from the egg trap.

Figure 1. Wooden sheets where BSF adults laid their eggs

Figure 2. BSF eggs

BSF Larvae Rearing Bin.

1. Rearing bin was made out of an empty plastic container (12 x 8.5 x 3) inches designed for BSF culture where a ramp and a trap container were provided for self-harvesting of the larvae (Figure 3). Bins were placed in a netted housing to prevent another type of flies from contaminating the feed substrate and egg deposition of wild Black Soldier Fly.
2. A total of 100 grams of BSF larvae (1,000 larvae) were introduced in the different experimental box in every life cycle period form March –August 2020.
3. Fruit and vegetable waste were collected from Dumalinao Public Market while manure was collected from breeder sow maintained at the station (Figure 5). 250 grams of waste substrates was given during the conduct of the study and replenish with 250 grams once a week as needed until more than 50% of larvae metamorphosed into prepupal.
4. For all treatments, weighing was done on every 3rd day by randomly counting 50 larvae from each container over a period of six (6) months or four (4) life cycles of black soldier fly larvae. The total weight obtained was divided by the number of larvae to obtain an average weight of each larva. The final prepupa weight was taken to be the average weight recorded on the day of harvest.
5. A temperature & humidity meter was placed inside the bin to monitor and control the temperature and relative humidity to prevent overheating of a pen. A temperature of 29-31℃ and relative humidity of 50-70% was maintained.

Figure 3. Rearing Bin

Figure 4. Seven (7) days old BSF larvae

Figure 5. Fruit Waste and Vegetable scraps from the Public Market

Breeding Cages

a. Self -harvested BSF larvae were collected and put in a plastic container filled with rice hulls. The container was covered with a net that has a mesh size that won't let flies through (Figure 6).
b. As some of the pre-pupae is close to become an adult they were transferred and placed inside the four (4) units (L-3.7 x W-3.7 x H-5 feet) rearing case made by net (Figure 7), where they are allowed to become adult flies, mate and lay eggs (Figure 8).
c. Containers filled with moist fermented feed and source of clean water were placed at the bottom to encourage laying of eggs. Using a rubber band, 4-5 pieces of wooden sheets with small gap between them will be placed inside as egg straps where fertile females would lay eggs. The feed will be kept moist all the time to avoid female laying directly on the food source.
d. Harvesting of the eggs and replacement of wooden sheet was done every day to make the empty flute space available for oviposition.
e. The newly hatched BSF larvae collected from the first cycle were used as an initial weight for the 2nd cycle period of BSF larvae production; the same procedure was used for rearing BSF larvae until the 4th life cycle period.

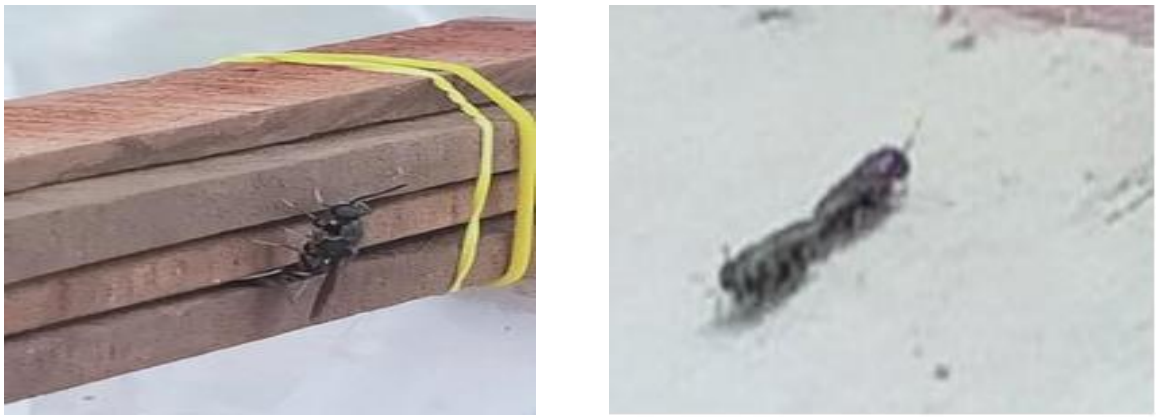


Figure 6. Pupae filled with rice hull

Figure 7. BSF rearing case

Figure 8. Oviposition & mating of BSF adults

Data Gathered:

1. Production Data of Black Soldier Fly
   a. Initial Weight

      A 4 days old BSF larva was considered as the initial weight and measured using an electronic weighing balance readable to 0.01 g. In order to ensure a uniform size and mass of the 1st instar larvae used in the experiment, they were sieved with a mesh diameter of 1.2 mm and only those that managed to pass through the sieve by themselves were used. 1000 pieces of BSF larvae weighing 100 grams were used per rearing bin.

   b. Final Weight

      Self-harvested larval wet weight was considered as the final weight of the larvae harvested in each treatment and was

measured using an electronic weighing balance readable to 0.01 g. Larvae migrated during 6th instar at 19-25 days after egg hatching or eclosion. The larvae that were not migrated to the container were harvested using sieving process (2 mm mesh diameter) to separate larvae and substrate residues.

c. Larva Period

Larvae Period of BSF is the feeding stage with five instars within 8-18 days. It was measured by counting the number of days from larvae to pre-pupae.

d. Pre-pupation Period

Pre-pupation covered until the pupation period. This is the stage where larvae stop feeding. They started migrated during 6th instar at 19-25 days after eclosion or egg hatching; they will look for a dry site and then stop moving until turns into adults. It was measured by counting the pre-pupation period until turns into fly.

2. Waste Reduction Rate (WR)

Waste reduction is the substrates residue that is not consumed by the BSF larvae during the feeding period.

It was estimated for each treatment based on the dry weight of the substrates using the formula. WR (%) = [1 − (Substrate residue/substrate added)] × 100

3. Average temperature

Temperature was gathered by placing thermometer in all experimental rearing bins per treatment during the larvae stage (2nd -5th instars) from 1st-4th cycle period; daily recording was done every noon time in order to properly monitor the room temperature in the area.

4. Nutrient analysis of larvae meal/treatment

A sample of 250 g larvae was taken from each treatment, sun-dried and submitted for analysis at Feed Chemical Analysis Laboratory, DA-Tumaga Complex, Zamboanga City.

5. Production Cost of Rearing Black Soldier Fly Larvae Fed with Different Waste Substrates

Results and Discussion

The larval and pupa stages of the Black Soldier Fly's life cycle are the longest, whereas the egg and adult stages are relatively short. The larvae stage lasts until the fifth instar, and the pre-pupation time (6th instar) varies depending on the waste substrates used. The newly emerging larvae begin feeding on control mixed feed substrates immediately for 7 days, then shift their diet till the pre-pupation stage begins.Table 1 presents the average production of black soldier fly larvae within four (4) life cycle period (6 months) fed with different waste substrates.

A total of 100 grams BSF larvae (1,000 larvae, 7 days old) were introduced in every experimental container.

Larvae period

The larvae period of BSF is the feeding stage with five instars. It was measured by counting the number of days from larvae to pre-pupae. Early maturation of larvae was observed within 18-19 days in fruit remains and T3-Vegetables scraps, while the larvae in swine manure substrates matured in 27 days.

Average pupation period

The life cycle of BSF larvae was measured by counting the pupation period until turns into an adult. This is the stage where larvae stop feeding during 6th instar. 90% of pupae from T1- fermented rice bran, T2-fruit remains, and T3-vegetable scraps reached the adult stage within 14-15 days.

Behavior of BSF larvae

The Black soldier fly (BSF) larval migration from plastic boxes to harvesting containers came down at night or in the early hours of the morning when it was still dark. Adult black soldier flies have greatly reduced sponging mouthparts and can only consume liquids such as flower nectar or do not eat at all.

Average final weight of BSF pupae

Larval wet weight was considered as the final weight of the larvae harvested in each treatment. The variation in the number of larvae harvested was attributed to the intensity and quality of odors produced by the decomposing food, as well as the nutritional quality of the substrate materials.

Table 1. Average production of black soldier fly larvae fed with different waste substrates from March-August 2020

Treatment	Initial Weight (grams)	Larvae Stage (days)	Pre-Pupation Period (days)	Final Weight (grams)
T1- Fermented rice bran (Control)	100.00	22.00 [b]	14.67 [b]	205.00 [b]
T2- Fruit remains	100.00	18.00 [c]	14.33 [b]	218.67 [a]
T3- Vegetable Scraps	100.00	19.00 [c]	14.33 [b]	214.00 [ab]
T4- Swine Manure	100.00	27.00 [a]	26.00 [a]	137.00 [c]
P value		<.001	<.001	<.001
CV%	**0.23**	**3.80**	**4.08**	**2.52**

Any two means having a common letter are not significantly different at the 5 % level of significance.

Average weight increment BSF larvae in mg fed with different waste substrates

Weighing was done on every 3rd day by randomly counting 50 larvae from each container. The sampled larvae were collectively weighed on an electronic scale and thereafter, returned to their respective feeding containers. The total weight obtained was divided by the number of larvae to obtain an average weight of each larva. The weight gain after every 3 days was calculated by comparing the obtained average larval weight with the previous mean larval weight.

Figure 1 shows the average weight increment of BSF larvae (wet) in mg larvae fed with different waste substrates. A significant effect was observed at the larvae weight increment fed with different waste substrates. The results showed the varied reaction of BSF larvae for the different substrates at different stages of growth. Quicker adaptation at some stage in the early growth of BSF larvae around 35-48 mg fed with various substrates, and later improved larval growth phase has been observed for fruit waste and vegetable scraps. In comparison, BSF larvae reared on swine manure showed the least increase during the last larvae period with 137 mg as compared to the 219-214 mg for fruit waste and vegetable scraps respectively. The findings indicated that BSF larvae had different substrate preferences depending on their growth stage. The structure of the feedstock can influence the substrate choice at a given stage of development. Furthermore, larval development and weight gain are definitely linked to the nutrient composition of the diet (Oonincx et al., 2015).

BSF emit chemicals that deter other flies from laying eggs on the same medium. They can aid in the management of housefly populations in livestock farms and residences with inadequate sanitation.

Waste reduction rate of BSF larvae fed with different waste substrates

Fruit waste and vegetable scraps were found to have higher reduction rates than swine manure in Black Soldier Fly Larvae (BSF) larvae. The texture of fruits and vegetable wastes, which are often watery and easy to decompose, could explain these findings.

Due to excessive heat or suffocation, several of the larvae fed fermented rice bran died. Rice bran has fine pores, a low density, but a high rigidity, which prevents oxygen from reaching the substrates.

Swine manure was found to have the lowest decrease rates of BSF larvae that could be attributed to its low energy content, as it is a pig digestion waste product. The energy content of a substrate can affect its reduction efficiency, according to a study by (Nguyen et al., 2011).

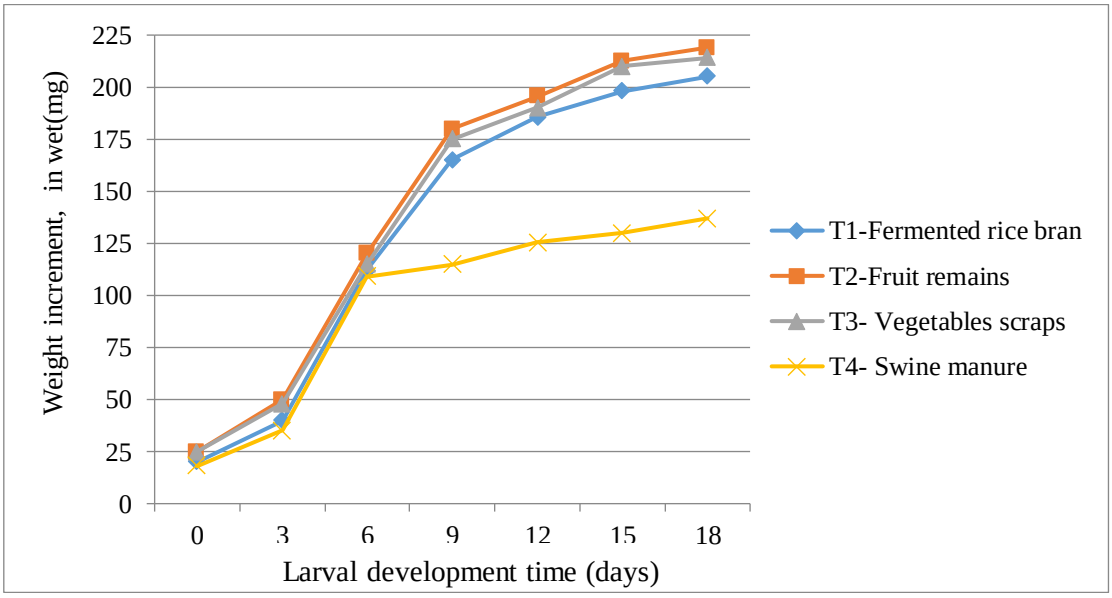


Figure 1. .Average weight increment of BSF larvae (wet) in mg fed with different waste substrates, 1st-4th cycle, CY 2020

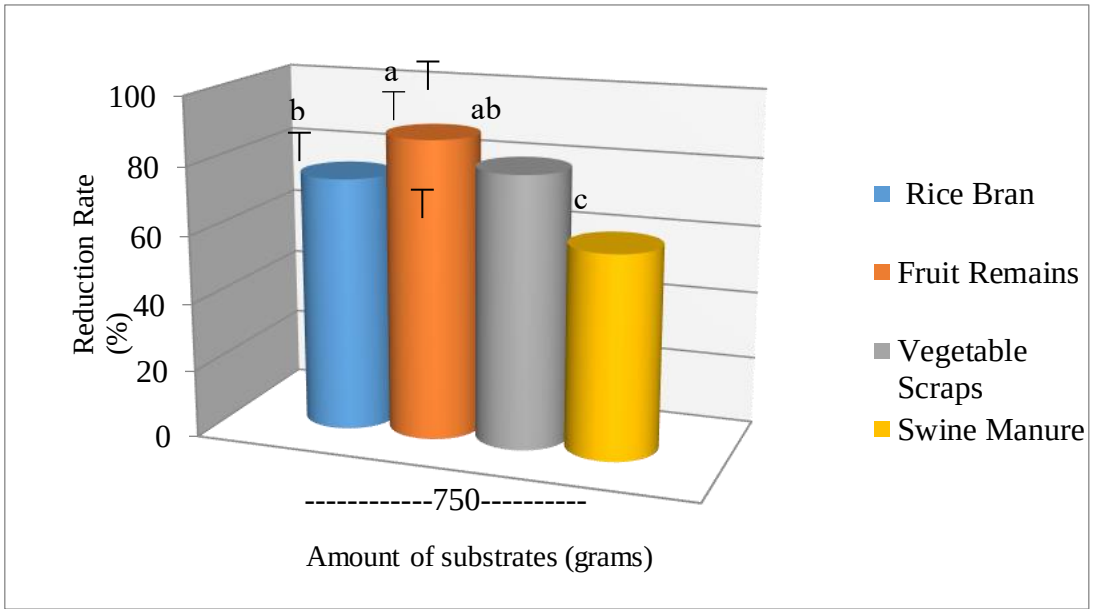


Figure 2. Average reduction rate of different waste substrates fed to black soldier fly larvae, CY-2020

The average temperature during the conduct of the study

Temperature plays a role in several important factors regarding insect farming. BSF larvae are very sensitive to the external environment such as temperature and rearing medium.

Figure 3 shows the average temperature (℃) at the rearing bin within the larvae stage fed with different waste substrates from March-August 2020. The temperature of the rearing bin from March-August 2020 ranged from 25-31 (℃) which is suitable for the required temperature needed for the duration of

the feeding period of the BSF larvae stage. This was also reported in a study that the precise temperature of BSF larvae is between 25-31℃ (Zhang et al., 2010). The temperature of rearing bin was gradually increased every 3 days; it was attributed to the process of decomposition of the waste substrates in which it produced heat while continuously decomposed.

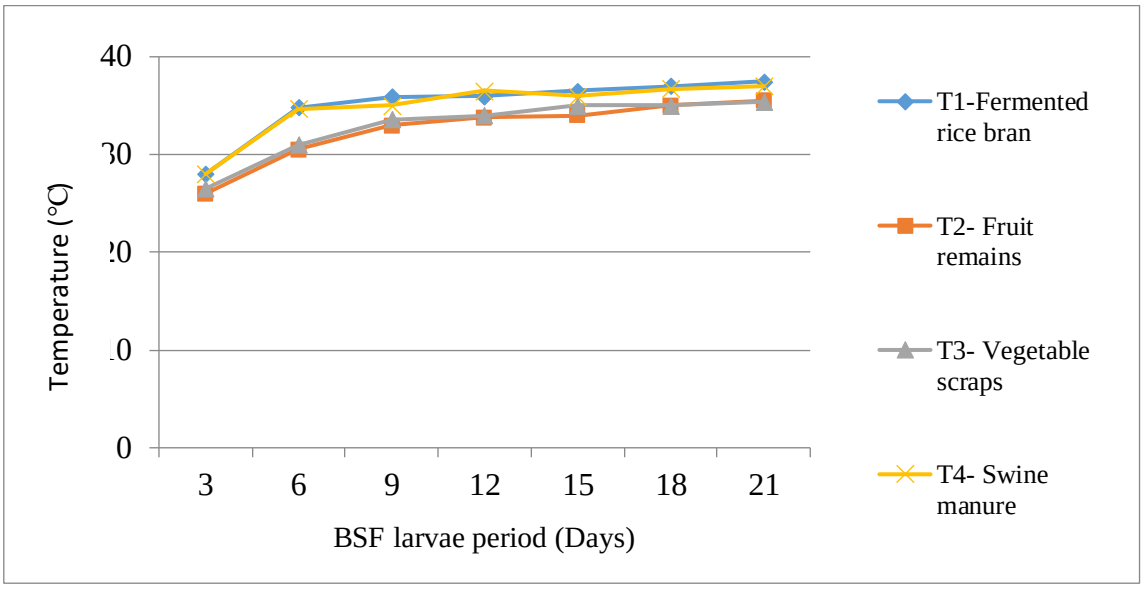


Figure 3. Average temperature (Celsius) on rearing bin fed with different waste substrates from March –August 2020 at Dumalinao, Zamboanga Del Sur

Nutrient analysis of BSF larvae

The nutrient content of BSF larvae especially crude protein is very important because it will be the basis whether it is comparable to the most commonly used protein sources like soybean oil meal and fishmeal. A ¼ kg of self-harvested larvae was collected, dried, and submitted for nutrient analysis.

The BSF larvae fed with fruit and vegetable substrates had crude protein content of 47 & 43%, respectively; in addition to other important nutrients compared to the 29.06% CP content of swine manure substrates. In general, a BSFL meal might be used to replace fish meal and soybean meal in animal nutrition.

Table 2. Chemical analysis of black soldier fly larvae fed with different waste substrates compared to fishmeal and soybean oil meal, CY-2020

	Parameters								
Treatment	**%CP**	**ME**	**%DM**	**%M**	**%Ca**	**%P**	**%Ash**	**Salt**	**Fat**
T2-Fruit remains	47.7	1,871.70	92.3	7.7	2.1	18.3	14.3	1.5	19.3
T3-Vegetable Scraps	43.8	1,851.90	96.3	3.7	16.4	13	7.2	2.2	32.2
T1-Rice bran (Control)	37.7	1,670.50	95.4	7.1	0.5	11.9	6.8	2.6	34.1
T4-Swine Manure	29.1	1,377.30	3.9	7.1	0.3	12	5.4	2.4	31.1
Fish meal [1]	60		92.5	7.5	79.3	39.8	32.5		
Soybean meal [2]	49		87.7	12.3	3.9	7.1	7.4		

Source: T1-T4 (Regional Animal Feed Analysis Laboratory, DA Region IX; CY 2020): [1] & [2] (Feedipedia, 2020)

Return on capital of rearing BSF larvae

Table 3. Return on capital of rearing black soldier fly larvae fed with different waste substrates from 1st -4th cycle BSF larvae period, CY-2020

Treatments	Market Value of BSF Larvae (100Php/ 100 grams)	BSF Larvae Harvest (kg)	Expenses (Php)	Gross Income (Php)	Net Profit (Php)	ROC (%)
T1- Fermented rice bran	100.00	1.23	914.50	1230	315.50 [c]	25.65
T2- Fruit remains	100.00	1.31	743.50	1310	566.50 [a]	43.24
T3- Vegetable scraps	100.00	1.29	743.50	1290	546.5 [b]	42.36
T4- Swine manure	100.00	0.80	743.50	800.00	56.5 [d]	7.06

Table 3 shows the return on investment for rearing Black Soldier Fly Larvae on various waste substrates. BSF larvae fed with fermented rice bran cost Php 833.50.00 and were produced using an egg hatchery, one plastic rearing bin, and a breeding enclosure filled with fruit and vegetable waste. Based on the study results, one plastic fattening bin measuring 12 x 8.5 x 3 inches can contain 100 grams/1000 BSF larvae and harvest 219 grams every cycle. During the pupation period (19-25 days from egg hatching), an open BSF system with a wide fattening bin measured (1 x 0.5 x 0.5) meter also used in the station will harvest more or less 4kg larvae fed with vegetables and fruit waste. Other similar results show that an open BSF system with a fattening bin of (1 x 0.5 x 0.5) meter will produce 3-4 kg during pupation (Nyakeri et al., 2016).

With the market value of 100 Php/ 100 grams BSF larvae, it will have an annual Return of Capital (ROC) to 43.42% and 42.54% fed with fruit waste and vegetable scraps respectively. It has a significant net profit, with Php 566.5 in BSF larvae fed with fruit remains compared to Php 56.6 in BSF larvae fed with swine manure. This ensures the sustainability of the production system because these are waste products and are locally available.

Conclusion

According to the study's findings, employing different waste substrates had a significant impact on the production and nutritional value of BSF larvae. Fruit and vegetable scraps produced the most BSF larvae, with crude protein contents of 47 and 43%, respectively. The BSF larvae's nutritional composition suggests that using fruit and vegetable waste as a protein replacement might be an affordable and sustainable option. Because the technique used in this study doesn't need a lot of labor, it may be used by local farmers who wish to increase the protein intake of their cattle.

Recommendations

Investigate the energy value of using larval black army flies instead of fishmeal or soybean oil meal as a protein source for small-scale swine and poultry production. Study the open method of raising BSF larvae for adoption by smallholder farmers in the context of the regional environment. To increase the black soldier fly's productivity and sustainability in semi-commercial swine and poultry production, establish a production/processing plant.

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