Growth and Nutritional Performance of Black Soldier Fly (Hermetia illucens) Larvae Reared in Fermented Rice Straw and Duck Manure

Kristopher Ray S. Pamintuan¹², Hanika Angel T. Agustin¹, and Ericka D. Deocareza¹

1 School of Chemical, Biological, and Materials Engineering and Sciences, Mapua University, Intramuros, Manila, Philippines
2 Center for Renewable Bioenergy Research, Mapua University, Intramuros, Manila, Philippines
E-mail: krspamintuan@mapua.edu.ph

Abstract. Black soldier fly larvae (BSFL) are known to convert organic wastes into useful biomass, of which the composition depends on the substrate. It is of interest whether feed protein can be sustainably obtained from waste materials by feeding them to BSFL. This study aimed to convert rice straw and duck manure into BSFL biomass for conversion of waste into animal feed. The growth parameters of BSFL fed with pure fermented rice straw, pure duck manure, and an equal parts mixture of the two as well as its nutritional composition was determined. The larvae’s efficiency to consume and convert the different substrates was also evaluated. Results showed that BSFL fed with duck manure had significantly higher average individual weight of 0.0619± 0.004 g, followed by mixture of duck manure and rice straw (0.0614± 0.001 g), while those fed solely with rice straw did not accumulate the same biomass (0.0415± 0.002 g). Correlations were also made for mass-length, mass-width, and length-width. Mass-length connection was the most reliable correlation (r = 0.732). The harvested BSFL protein was the highest for those fed with rice straw at 34.62%. Feed conversion ratio ranging from 3.71 to 11.3 was achieved for the substrates used. The availability of the waste substrate in large quantities coupled with efficient biomass conversion makes BSFL a sustainable organic matter converter primarily useful as additive to animal feed.

1. Introduction
The rising cost of commercial animal feed is of great concern in the Philippines, a primarily agricultural country. The scale of production cost increases with the rising population causing higher demand for animal protein, hence demanding more animal feed. An estimated 1.3 billion tons of grain is required by 2050, 40% of which would be consumed for feeding livestock [1]. To offset feed production costs, it is imperative that suitable replacements for grain as animal feed are developed without affecting the quality of the animal’s meat. Formulating substitute feed that have substantial amounts of protein and fat is of priority. Black soldier fly (Hermetia illucens) larva (BSFL) is a promising alternative source of feed. BSFL, as voracious feeders, utilizes waste biomass that can no longer be used as feed and convert it to its own biomass which can then be a rich source of protein [2]. The production of BSFL meal reared on different types of waste and organic materials upon conversion produce protein-rich and fat-rich biomass that can be used solely or supplementing part of animal feed.
BSFL has a high nutritional value to animals due to a good protein to fat ratio averaging 42% protein and 35% fat depending on feed [3]. BSFL having detritivore qualities only consume decaying matter such as decomposing plants, animal parts, and manures which makes it a well-known bio-recycling organism [4]. A number of studies made use of different substrates in rearing black soldier fly larvae. A study using poultry manure in rearing BSFL resulted to 37.9% protein and 18.73% fat [5]. A higher percentage of nutrients were obtained by utilizing swine manure, 43.2% protein and 33.1% fat were extracted [6]. Majority of previous studies utilized manures and other animal-based wastes. The Philippines, being an agricultural country, produces large amounts of cellulosic agricultural wastes, primarily, rice straw as by-product of rice production. It also boasts a growing backyard duck farm industry producing large amounts of duck manure as waste. It is of interest whether these waste substrates can be utilized by BSFL and turn them into useful feed biomass.

The main objective of this study is to characterize the growth parameters of BSFL reared on duck manure, fermented rice straw, and its mixture. Additionally, the nutritional composition of produced BSFL as well as its bioconversion ratios were analyzed. Rice straw is notoriously difficult to digest for some livestock due to its cellulosic nature, so bioprocessing it to be more digestible and palatable can be a useful tool for waste management. This study provides useful information regarding the growth rate of BSFL that can be used for computational models and may aid in designing large-scale BSFL rearing facilities.

2. Methodology

2.1. Materials
The duck manure and rice straw were obtained from a farm situated in Candaba, Pampanga, Philippines. The BSF eggs were bought from a small backyard farm in San Pablo, Laguna, Philippines. Approximately 3 kg of duck manure were gathered and sun-dried to remove moisture and to manage the scent of the manure. The rice straw was chopped into 1-inch segments and was soaked in tap water until waterlogged. The rice straw was fermented in a covered container for a week before it was dried, weighed, and portioned for feeding. All feed materials were collected in bulk and mixed together to ensure homogeneity. Each feed was portioned out at 3 grams per cube and were covered with plastic wrap and stored in the freezer. The feed were defrosted 30 minutes before every feeding time.

2.2. Cultivation
Nine rectangular acrylic containers were constructed as the cultivation quarters of the larvae (Fig. 1). Each container represented a trial under three experimental treatments: larvae fed with pure duck manure (DM), pure rice straw (RS), and mixed feed of duck manure and rice straw having 1:1 ratio (DM-RS). Upon hatching, the larvae were nurtured at the hatching bins and were fed with duck layer pellets for 7 days. After the 1-week growth period, the larvae were separated into three experimental groups mentioned above.

The cultivation bins were weighed before manually placing 100 cultured 7-day-old black soldier fly larvae in each area. The estimated time for this cultivation process was 2 to 3 weeks before the larvae are set for pupation. The cultivation bins were covered with plastic wrap riddled with tiny holes for gas exchange and to further prevent any larva from escaping. The bins were also isolated so that ants will not be able to reach the larvae. Three groups of feed were prepared for the experiment (DM, RS, and DM-RS) composing of three trials for each group. For the first few days, 6 cubes of feed were defrosted and were given to each of the group. Another set of feed was added to the cultivation bin after the larvae fully consumed the previous feed. The time of full feed consumption for every set-up was also observed.
2.3. Data gathering
The relative humidity and temperature were recorded daily. These parameters are crucial at each life stages of the black soldier fly larvae. During the entire experimental period, no sudden fluctuations in both temperature and relative humidity were recorded.

The length, width, and weight of the larvae were manually measured daily in the morning before feeding. At each cultivation bin, 20 random larvae samples were measured to obtain the data. The mass of the remaining feed was measured after harvesting the larvae. This will help with monitoring and determining the feed intake of the larvae for the duration of the experiment. The feeding time of the larvae was every morning after the determination of the relative humidity and temperature, and after taking body measurements.

After majority of the larvae are about to pupate (>95%, characterized as a period where the larvae have darkened and are less mobile), the larvae were harvested and frozen overnight at -5°C. The frozen larvae were then dried at 80°C until constant weight and were pulverized to a powder. Samples were sent to a laboratory (FAST Laboratories, Philippines) for analysis of crude protein, crude fat, ash, and carbohydrates.

3. Results and discussion

3.1. Growth kinetics
The growth of the larvae was highly influenced by the feed. The growth of BSFL reared on different substrates were modelled using Gompertz equation (Equation 1).

\[ m(t) = c_1 \exp[-c_2 \exp(-c_3 t)] \]  

(1)

In order to determine the constant values (a, b, c) of Equation 1, the actual mass from the recorded data were plotted against time. This method allows for a more thorough analysis of the growth pattern of the larva. The Gompertz constants obtained are shown in Table 1.

| Feed           | \( c_1 \)  | \( c_2 \)  | \( c_3 \)  |
|----------------|-----------|-----------|-----------|
| Duck Manure    | 0.06415   | 17.49     | 0.4488    |
| Rice Straw     | 0.04226   | 2226      | 1.334     |
| Mixed Feed     | 0.06005   | 3.113     | 0.2311    |

The growth pattern of BSFL reared in different substrates are shown in Figure 2.
The growth rates of the larvae were determined by obtaining the first derivative of the Gompertz equation as a function of time as mathematically presented in Equation 2. Subsequently, Figure 3 illustrates the growth rate of larvae fed with different feed.

\[
\frac{dm}{dt} = c_1 c_2 c_3 \exp[-c_2 \exp(-c_3 t) - c_3 t]
\]  

(2)

When evaluating growth rate curves, sharp peaks are to be avoided as those signify a rapid growth spurt which is followed by stagnation. That is what happened to the larvae fed with rice straw. The growth rate of the larvae reared on a mixed feed of duck manure and rice straw performed the best as the growth is sustained over a larger period. The addition of duck manure to fermented rice straw...
made some parts of it bioavailable for larvae. However, on some cases, it has been observed that the larvae are selectively eating the duck manure, leaving the rice straw untouched. It is worth noting that larvae fed with DM and DM-RS reached about the same final pre-pupal weight, but at different paces. A recommendation for future studies would be to finely grind the rice straw prior to feeding to avoid such cases.

![Figure 3. Growth rate curves of BSFL reared in DM (**), RS (—), and DM-RS (---)](image)

The average final pre-pupal weights of larvae before harvesting are shown in Table 2. The average pre-pupal weight is highly influenced by the different substrates fed. BSFL fed with duck manure and mixed feed equally gave higher average pre-pupal weights of 0.0619±0.004 g and 0.0614±0.001 g, respectively. Larvae reared on rice straw obtained the lowest average pre-pupal weight of 0.0415±0.002 g which is significantly lower (α = 0.05) compared to the other two substrates. Similar trends were also observed for the average length which can be supported by correlation coefficient values given in Table 3, in which a direct relationship is established between the mass and length of the larva. There are no significant differences found in the average width of the larvae fed on different substrates. These results further support the earlier findings that rice straw alone is a poor substrate for BSFL, but if mixed with duck manure, it can be utilized to produce more BSFL biomass.

| Feed   | Prepupal mass (g) | Length (mm) | Width (mm) |
|--------|-------------------|-------------|------------|
| DM     | 0.0619±0.004      | 11.29±0.40  | 3.62±0.14  |
| RS     | 0.0415±0.002      | 9.51±0.60   | 3.39±0.14  |
| DM-RS  | 0.0614±0.001      | 11.23±0.62  | 3.54±0.09  |

3.2. Parameter correlations

Pearson’s correlation coefficient \( r \) was used to determine what growth parameters of BSFL are the most linearly related. Correlations were tested for mass-length, mass-width, and width-length. Fitting constants as well as values of correlation coefficient are given in Table 3.

| Feed   | Width-Length | Mass-Length | Mass-Width |
|--------|--------------|-------------|------------|
|        | \( a \)     | \( b \)     | \( r \)    | \( a \)     | \( b \)     | \( r \)    | \( a \)     | \( b \)     | \( r \)    |
| DM     | 0.2056       | 1.427       | 0.5935     | 0.0089      | 0.0460      | 0.7817     | 0.0156      | 0.0004      | 0.4986     |
| RS     | 0.265        | 0.8861      | 0.6247     | 0.0060      | 0.0194      | 0.6385     | 0.0092      | 0.0085      | 0.4717     |
| DM-RS  | 0.2308       | 1.114       | 0.6521     | 0.0080      | 0.0370      | 0.7765     | 0.0106      | 0.0146      | 0.4168     |
Overall, mass-length correlations appear to be the strongest ($r_{ave} = 0.732$). This is generally true for insects, both in the larval and adult stage [7]. These relationships allow for the estimation of length and width of BSFL being fed with DM, RS, and DM-RS at any length of time by combining parameter correlations with the developed Gompertz curve above.

3.3. **Bioconversion ratios**

The computations for overall degradation (OD), waste reduction index (WRI), efficiency of conversion of digested feed (ECDF), feed conversion ratio (FCR), and survival rate (SR) (Equations 3-7) were performed to determine the efficiency of the black soldier fly larvae in transforming waste substrates into protein-rich biomass.

\[ OD = \frac{\text{total feed consumed (g)}}{\text{total feed given (g)}} \]

\[ WRI = \frac{\text{number of larvae} \cdot \text{rearing duration (d)}}{\text{change in larval mass (g)}} \]

\[ ECDF = \frac{\text{total feed consumed (g)}}{\text{change in larval mass (g)}} \]

\[ FCR = \frac{1}{ECDF} \]

\[ \text{Survival rate} = \frac{\text{final larval count}}{\text{initial larval count}} \]

### Table 4. Summary of bioconversion ratios of BSFL

| Parameters                              | DM     | RS     | DM-RS  |
|-----------------------------------------|--------|--------|--------|
| Rearing Duration (d)                    | 22     | 22     | 22     |
| Overall Degradation (%)                 | 37.0±4.5 | 25.4±2.6 | 36.2±8.0 |
| Waste Reduction Index (mg/larva-d)      | 3.14±0.38 | 2.16±0.22 | 3.07±0.68 |
| Efficiency of Conversion of Digested Feed (g/g) | 0.269 | 0.089 | 0.266 |
| Feed Conversion Ratio (g/g)             | 3.71   | 11.3   | 3.76   |
| Survival Rate (%)                       | 79.6±20 | 73.9±7.5 | 76.2±4.4 |

The larvae were cultivated for a total of 22 days on the experimental substrates. The larval survival rate was not significantly different for all the substrates ($\alpha = 0.05$). The larvae reared in pure duck manure performed the best in terms of all the bioconversion ratios: high OD, high WRI, high ECDF, and low FCR (Table 4). Larvae reared in pure rice straw showed the extreme opposite, which indicates that rice straw alone as a feed is not good for BSFL. Less of the substrate is being consumed by the larvae, and even less of the consumed part is being converted into useful biomass. The obtained ECDF of larvae fed with rice straw is in agreement with a previous study [8]. The addition of duck manure to fermented rice straw significantly improved the bioconversion ratios, further strengthening the evidence gathered previously that supplementing duck manure to rice straw can make it palatable and more available for the larvae.

3.4. **Proximate analysis**

The proximate analyses of harvested larvae are summarized in Table 5.

### Table 5. Proximate Composition of Black Soldier Fly Larvae Reared on Different Substrates

| Parameters          | DM     | RS     | DM-RS  |
|---------------------|--------|--------|--------|
| Crude Protein (%)   | 18.9   | 34.62  | 20.0   |
| Crude Fat (%)       | 0.673  | 8.00   | 0.816  |
| Carbohydrates (%)   | 29.527 | 39.45  | 32.022 |
| Ash (%)             | 50.9   | 17.92  | 46.8   |
Several studies presented that BSFL contain an average of 42.1% crude protein, 34.8% ether extract (lipids), 14.6% ash, 7.9% moisture, 7.0% crude fiber, 5% calcium, 1.5% phosphorus, and 1.4% nitrogen free extract (NFE) [2]. The larvae reared on duck manure obtained 0.673% of crude fat, 18.9% crude protein, 50.9% ash, and 29.537% carbohydrates. The larvae in this experimental feed have the highest amount of ash and the lowest amount of fat. One factor that might explain this observation is that the larvae only ate 37.03% of the total substrate given for 29 days of rearing period. The fat in the system of the BSFL was converted to energy since they are not consuming the right amount of feed for their growth, hence the obtained low amount of fat. Various studies have showed high amounts protein and fat content for larvae reared on animal manure. Larvae reared on pig’s blood and manure have 35.90% crude protein, 6.50% crude fibre, and 7.80% ash [9]. Larvae reared on poultry manure generated 37.9% protein and 19.73% fat [5]. The duck manure that was used as substrate had undergone drying process, the protein and fat content of the larvae may be higher if the duck manure fed was in fresh and newly harvested condition. Another factor is the moisture content of the feed as larvae may not eat if the moisture content of the feed is too low. The larvae reared on fermented rice straw obtained the highest values of nutrients, which was unexpected. The high amount of moisture containing the fermentation broth adhering on the rice straw is one factor that generated the increased amounts of crude protein, crude fat, and ash. The mixture of duck manure and rice straw have 20% crude protein, 0.816% crude fat, and 46.8% ash. It is important to recognize that the amount of fat accumulated depends on the type of the feed fed to the larvae as well as the rearing duration. The black soldier fly larvae lifespan is dependent both on environmental conditions and the type of feed; it is worth noting the BSFL fed with rice straw took longer to mature with virtually no increase in mass (around 30 days), while BSFL fed with manure and the mixed feed matured earlier (20-22 days) but was cultured at the same duration as the larvae fed with rice straw. This makes the larvae fed with rice straw concentrate its nutrients while the others ended up using its own stores of nutrients. Also, maturing BSF larvae are known to experience decreases in fat content as they mature [10].

4. Conclusions

Conclusively, the results of this study suggest that BSFL can convert duck manure and fermented rice straw into valuable biomass, with varying rates and efficiencies. The BSFL reared on pure rice straw contains more protein and carbohydrates than the other two substrates but it grows slowly, suffers from poor bioconversion, and attains a lower pre-pupal mass. Larvae reared on a mixture of rice straw and duck manure performed better overall, suggesting that a low-value waste such as rice straw can be converted to useful animal protein by simply adding some duck manure to make it more available for the BSFL. A cultivation system of BSFL reared on fermented rice straw can deliver a high-quality insect resource with further development and refinement.

5. References

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