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Research Article

Growth and Development of Black Soldier Fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) Larvae Grown on Carbohydrate, Protein, and Fruit-Based Waste Substrates

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ABSTRACT

There has been a surge in interest in using food waste (FW) as an insect-rearing substrate in recent years. We examine the effect of protein-based food waste (leftover boneless chicken, LBC), carbohydrate-based food waste (overnight rice, OR), and fruit-based food waste (rotten banana, RB) on the following parameters: substrate reduction (SR), waste reduction index (WRI), bioconversion rate (BCR) and relative growth rate (RGR). BSFL reared on OR feed substrate had the highest biomass (0.23±0.01) g at d_9 followed by RB (0.22±0.00) g and LBC (0.16±0.00) g. Larvae from OR-fed BSFL were the longest, averaging 20.53±0.46 mm in length on d_{12}. The SR calculated for all feed substrates were as followed: RB (95.35 ± 0.33)% > OR (85.29 ± 0.80)% > LBC (83.17 ± 0.27)%. The WRI for control (C) and BSFL-fed on all feeds were in the following manner: (RB_C:8.90±0.00 g days^{-1}, RB_{BSFL}:9.53±0.00 g days^{-1}) > (OR_C:7.35±0.00 g days^{-1}, OR_{BSFL}:8.53±0.09 g days^{-1}) > (LBC_C:6.90±0.00 g days^{-1}, LBC_{BSFL}: 8.32±0.03 g days^{-1}). OR-fed BSFL showed the highest BCR (76.0±1.0) % and RGR (0.32±0.01) days^{-1}. The FW's self-composting (as in control) influenced the BSFL's SR and WRI in all diets. Overall, the BSFL's growth and development are affected by the nature, quality, and type of diet of the feed substrates.

Key words: Black soldier fly larvae, carbohydrate, fruits, protein, valorization

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INTRODUCTION

Nearly every country in the world is grappling with the problem of food waste (FW). Municipal solid waste (MSW) has grown to be a major environmental problem in most major cities, especially in developing countries. In low-income countries, FW makes up 90% of MSW, and it accounts for 44% of global MSW (Kaza *et al*., 2018). In 2016, FW accounted for 45% of the MSW in Malaysia, where it amounted to around 16,688 tons per day (Sharif, 2018). Based on the annual population growth rate, solid waste generation is expected to exceed 31,000 tonnes per day by 2022 (Cheng *et al*., 2017). The use of landfills is widespread in Malaysia, as it is in many other countries around the world. As of 2016, only 15% of Malaysia's 157 operational landfill sites were sanitary landfills (Jayanthi *et al*., 2016). Non-sanitary or poorly engineered landfilling endangers the surrounding environment by polluting the air, water, and soil (Choon *et al*., 2017).

There has been a lot of focus on the development of new and improved waste recycling processes, some of which include rapid, clean, sustainable, and cost-effective recycling (Bigdeloo *et al*., 2021). A circular economy is a viable option for reusing waste resources due to a major increase in waste disposal. The valorization of FW materials into value-added goods has garnered considerable attention. Utilizing the intrinsic value of organic waste could assist to alleviate environmental consequences while also supplying food, fodder, and other commercially useful goods (Lalander *et al*., 2019). In Malaysia, FW valorization for livestock feed and organic fertilizer has been practiced sparingly (Woon *et al*., 2021). Malaysia's Feed Act (Act 698) prohibited the use of all FW as animal feed (Woon *et al*., 2021) since certain illnesses such as foot-and-mouth disease, swine fever, highly pathogenic avian influenza, and mad cow disease have been documented as being transmitted from food during the valorization process (Shurson, 2020).

Alternatively, organic wastes, especially FW, can be regarded as a valuable resource that can be transformed into 'wealth' through the bioconversion process (Diener *et al*., 2011). FW may be used as a compost material due to its ease of decomposition, leading to a better understanding of composting management as a result of environmental consciousness (Wang *et al*., 2021). The employment of saprophytic insects (e. g. black soldier fly; *Hermetia illucens*) in the treatment of organic solid waste has lately caught the public's attention. The larvae of the black soldier fly (BSFL) are ferocious feeders on decomposing organic material and could be used as the protein source in animal feed. BSFL possesses the capacity to minimize mycotoxin buildup as well as to suppress harmful microorganisms in the environment (Deng *et al*., 2020). A substantial amount of organic waste was reportedly ingested and digested by BSFL, with degradation rates as high as 70% (Diener *et al*., 2011). New-style biowaste recycling technology such as BSFL processing can provide marketable, valuable goods that can contribute to long-term financial success (Diener *et al*., 2011).

Composting by BSF larvae can add value to these wastes, by converting them into insect products potentially suitable for various applications (Isibika *et al*., 2021). According to earlier studies on BSFL, the larva from this species have the potential to convert organic wastes into marketable insect meals, with crude protein concentrations of 40% or less in full-fat meals and more than 50% when partially defatted (Surendra *et al*., 2016; 2020). To date, recent research has looked at the relevance of numerous macronutrients in feedstocks, particularly crude protein and non-fiber carbohydrate levels and ratios (Barragán-Fonsec, 2018). The purpose of this study is to examine the growth of BSFL utilizing three distinct dietary substrates: protein-based, carbohydrate-based, and fruit-based (representing a mix of protein and carbohydrate-based). Controlling BSFL by various types of food waste streams should assist in maximizing the waste reduction and the bioconversion of these materials into insect biomass (i.e., protein and fat). Therefore, the second objective of this study is to determine the relative growth rate, bioconversion rate, and substrate reduction of waste and waste reduction index.

MATERIALS AND METHODS

Adult fly colony

The primary black soldier fly (BSF) *Hermetia illucens (L.)* (Diptera: Stratiomyidae) colony was sourced from Entomal Biotech Sdn. Bhd. The colony was reared and maintained at room temperature (23 – 30 °C) in UiTM Insectarium throughout the study progress.

Diet source

Three different types of closed waste bins were provided in the UiTM Cafeteria for students to separate the waste categories by the feed substrates required for this study. The feed substrates being tested were the leftover boneless chicken (LBC), overnight rice (OR), and rotten banana (RB). The substrates were assembled and used as promptly as possible to reduce the risk of contamination by houseflies and other saprophagous insects. The feed substrates were ground and blended before use.

Experimental design

The BSF was reared in a 0.6 × 0.6 × 1.5 m cage and was observed for oviposition (Figure 1). An egg trap is placed in the cage to facilitate the egg collection process (Figure 2). The BSF eggs were collected after two days and placed in a 0.1 L plastic container until the emergence of BSF larvae. The larvae were fed chicken bran up until day five to produce a uniform size of 5-day-old larvae. Twenty larvae were then handpicked and weighed to calculate the average mass. A total of 8 g (± 200 5-day-old BSF larvae) were used for each treatment. Food waste feed consisting of rotten bananas (RB), leftover boneless chicken (LBC), and overnight rice (OR) was used for each treatment, and the experiment was conducted in triplicates.

The feed substrates were ground and blended before use. First, the larvae, with an average weight of 0.04 g/larvae, were placed on top of the prepared feed (150 g per treatment) in a plastic feeding container (15 × 5 × 13 cm), covered by a 2 × 2 cm holes lid to provide ventilation. The feeding container was subsequently put in a naturally ventilated and shaded environment, and the moisture content of all feed substrates was kept at 70% during the experiment. BSFL were reared on LBC, OR, and RB feed substrates at room temperature (23 – 30 °C) for fifteen days. Food rations were prepared, weighed, and kept frozen for 24 h before being used. The initial and final weight of each waste was recorded

Fig. 1. The black soldier flies breeding cage

Fig. 2. The black soldier flies eggs trap

before and after experimenting. Ten samples of BSFL were separated from each container on days 3, 6, 9, 12, and 15 for the measurement of the length and weight of the BSFL. The larvae were returned immediately to the container after the measurement. The weight and length measurements were conducted manually using a weighing balance and a ruler. The initial (day 0) and final (day 15) weight of each waste was recorded before and after the experiment to calculate substrate reduction percentage and waste reduction index (WRI). The weight of waste for all feed substrates devoid of BSFL was also noted as a control. After the experiment ended, the harvested BSFL was fed to the fish in the UiTM Recreational Lake.

Data analysis

The growth performance of BSFL rearing on different substrates was measured based on the weight and length of the larvae from 5-day-old to 20-day-old (at day 15). The BSF larvae start to lose their weight and length and turn into pre-pupae after 15 days of the experimental period. The SPSS statistical software for Microsoft Windows (version 27, IBM Corp., Armonk, NY, United States) was used for the statistical analyses. Shapiro Wilk test was first performed to assess the normality of the samples and the result was not significant. Therefore, One-Way ANOVA was used to compare the mean differences between the groups (LBC, OR, & RB). Additionally, the following equation was used to calculate the relative growth rate (RGR) and bioconversion rate (BCR) of BSFL (Cai *et al.*, 2019):

$$\text{Relative growth rate}(\text{days}^{-1}) = \frac{WI_f - WI_0}{t \times WI_0}$$

$$\text{Bioconversion rate }(\%) = \frac{WI_f - WI_0}{S} \times 100$$

Where; Wl_f is the weight of larvae (at the end of the experiment), Wl_0 is the weight of larvae (at the start of the experiment), *S* is the total quantity of substrate fed to the BSFL throughout the experiment and t is bioconversion time (day).

The weights of the feeding substrates and their residue were utilized to calculate the percentage of substrate reduction (SR) (Nyakeri *et al*., 2017) and waste reduction index (WRI) (g/day) (Raksasat *et al*., 2021). The WRI of each substrate was compared statistically to the control using a T-test. The calculation of SR and WRI were as follows:

$$\text{Substrate reduction(\%)} = \frac{\text{Total feed(g)} - \text{Residue feed (g)}}{\text{Total feed (g)}} \times 100$$

$$\text{Waste reduction index(g/day)} = \frac{\text{Total feed(g)} - \text{Residue feed (g)}}{\text{Rearing duration (day)}}$$

RESULTS

Larvae growth and development reared on different substrates

BSFL were effectively reared on all evaluated diets. The feeding experiment was observed for a maximum period of 15 days for LBC, OR, and RB as the diets, resulting in 20-day-old BSFL at the end of the experiment. The initial measurement of BSFL means length on day 0 was 7.0 mm. As shown in Figure 3a, the larvae reared on RB feed substrates attained their maximum mean length development on day 9 which was 20.47 mm. Meanwhile, for OR-fed BSFL, the maximum mean value was recorded on day 12 (20.53 mm). LBC-fed BSFL showed the highest consumption duration needed to attain its highest mean larval length of 20.13 mm which was on the final day of the rearing experiment (day 15).

Figure 3b shows the weight (g) development of BSFL reared on all three types of food waste substrates. On day 3 sampling, the weight for LBC, OR, and RB-reared BSFL were 0.10 g, 0.07 g, and 0.08 g respectively. Day 6 sample's revealed minimal differences in BSFL weight across all substrates. The weight percentage increment of BSFL from day 3 was 100%, 64%, and 30% for OR, RB, and LBC respectively. OR-fed BSFL attained its maximum weight on day 9 (0.23 g). Both LBC- and OR-fed BSFL achieved their highest larvae weight on the final day of the experiment which was 0.16 g and 0.22 g respectively. Overall, BSFL reared on OR had the highest larvae weight and consumed feed for a shorter amount of time than those put on alternate feed. The summary of the optimum larval length (mm) and weight (g) for LBC, OR, and RB are tabulated in Table 1.

The maximum BSF larval length and weight obtained from this study were shown in Table 1. BSFL had the longest length after being reared on OR (20.53 mm). BSFL reared on LBC had the shortest larval length (20.13 mm) followed by RB (20.47 mm). However, the *p*-value of the One-Way ANOVA's test was not significant indicating that the length of BSFL was not influenced by the types of rearing substrates $F(2,6) = 0.601$, $p = 0.601$. All the three-substrate produced ±20 mm of BSFL length during their optimum growth. In contrast to length, there was a statistically significant difference in BSFL weight rearing on the different types of substrates $F(2,6) = 187.61$, $p=0.01$. Further statistical analysis was performed using Tukey posthoc test. The result found that the *p* value of LBC and OR was 0.01 indicating the difference in larvae weight obtained from both substrates. The highest BSFL weight was produced by OR (0.23 g), while RB produced the second highest 179 (0.22 g). LBC produced the lowest increment of BSFL weight (0.16 g).

Bioconversion rate and relative growth rate of BSFL

Table 2 showed the BCR and RGR values for the LBC, OR, and RB substrates. The findings show that the BSFL converted OR (76.0%) slightly better than RB (72.0%) and LBC (48%) respectively. The RGR values for BSFL fed on all substrates based on the lowest to the highest rate were LBC (0.20 $days^{-1}$), RB (0.30 $days^{-1}$), and OR (0.32 $days^{-1}$). The OR-fed BSFL had the highest RGR value of all the samples tested.

Substrate reduction (SR) and Waste reduction index (WRI)

In this study, the percentage of the SR varied depending on the feeding substrates (Table 3). The SR was found to be in the range of 83% to 95%. RB (95%) had the highest SR, followed by OR (85%) and LBC (83%). This value was higher compared to Control RB (90%), Control LBC (64%), and Control OR (74%) respectively. This indicates increased decomposition activity when BSFL is present. As for the WRI, the control LBC (6.9 g/day) and control OR (7.4 g/day) were 17% and 14% lower than composting LBC and OR with BSFL (8.3 - 8.5 g/day). However, only a 7% difference in WRI was calculated between RB-control (8.9 g/day) and RB-fed BSFL (9.5 g/days).

DISCUSSION

Food waste poses a severe challenge to the viability of our food systems. Researchers, policymakers, non-governmental organizations, and the food industry continuously develop innovative, comprehensive solutions that are assessed and implemented to solve the problem of food waste (Ojha & Bußler, 2020). One of the efficient ways for handling food waste is the Conversion of Organic Refuse by Saprophagous (CORS) methods, which use decomposer insects like the BSFL to handle organic waste. The BSFL can quickly and

Table 1. The maximum larval mean length and weight of BSFL reared on different food waste for fifteen days

Feeding substrates	Maximum larval length (mm)		Maximum larval weight (g)	
	Mean	SD	Mean	SD
LBC	20.13	0.23	0.16	0.00
OR	20.53	0.46	0.23	0.01
RB	20.47	0.70	0.22	0.00

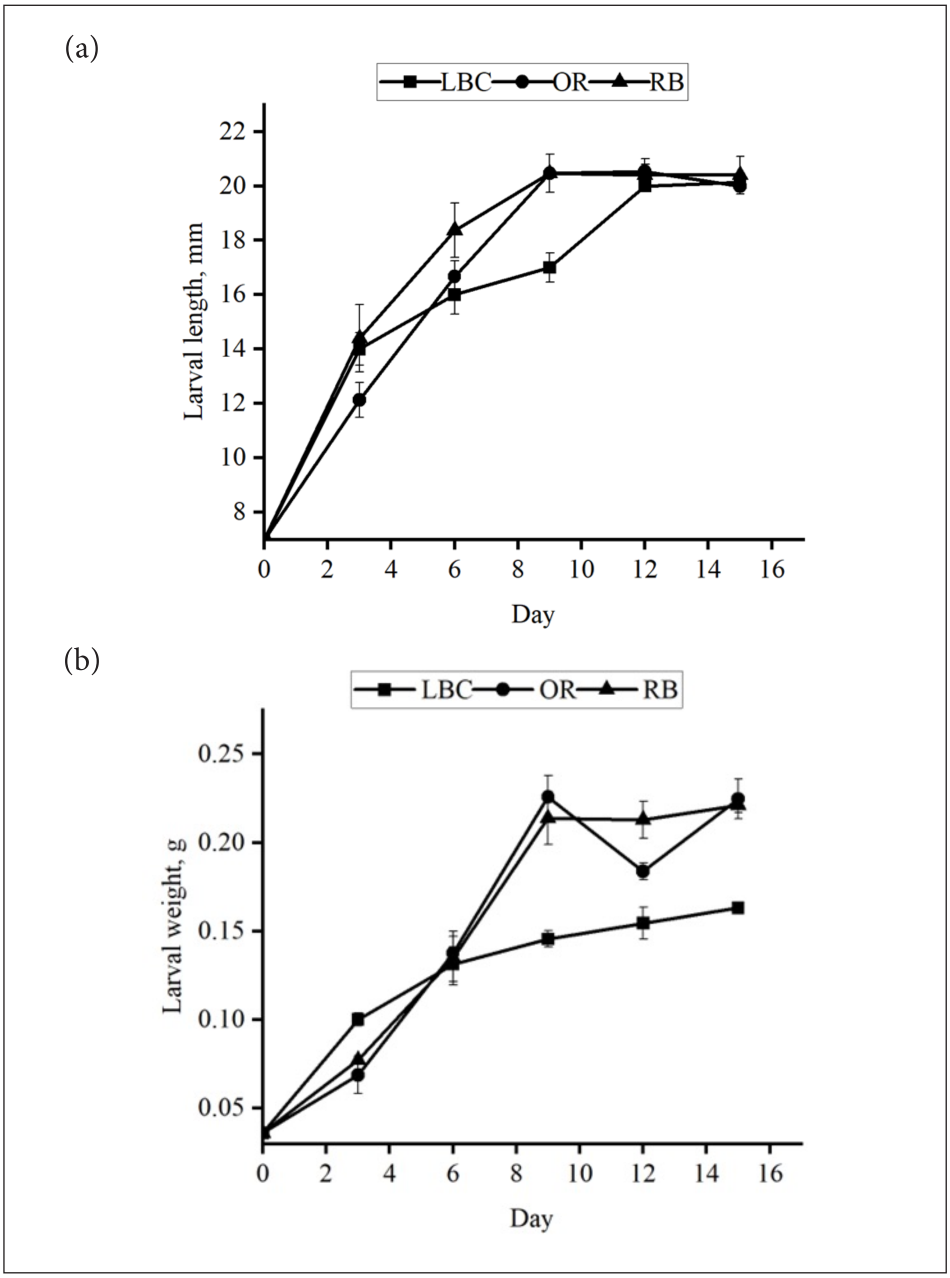

Fig. 3. The effect of diet source on the larval (a) length and (b) weight development of BSFL (Error bar = standard deviation)

Table 2. Bioconversion and relative growth rate of BSFL growth on different substrates

Type of substrates	Relative growth rate, RGR (day^{-1})	Bioconversion rate, BCR (%)
OR	0.32 ± 0.01	76.0 ± 1.0
RB	0.30 ± 0.03	72.0 ± 0.1
LBC	0.20 ± 0.02	48.0 ± 0.1

Table 3. Percentage Substrate reduction (SR) and Waste Reduction index (WRI) of different substrates

Type of substrates	Substrate reduction, SR, %	Waste reduction index, WRI (g/days)
RB	95.35 ± 0.33	9.53 ± 0.03
Control RB	89.72 ± 0.02	8.90 ± 0.04
OR	85.29 ± 0.80	8.53 ± 0.09
Control OR	73.50 ± 0.02	7.35 ± 0.04
LBC	83.17 ± 0.27	8.32 ± 0.03
Control LBC	69.41 ± 0.02	6.94 ± 0.03

effectively digest defective produce, garbage, crop waste, and trash from commercial food processing facilities (Hopkins *et al*., 2021).

Various waste organic materials are reduced and transformed by BSFL feeding on organic substrates derived from plants and animals (Diener *et al*., 2009). BSF primarily feeds during its larval stage and accumulates enough fat to diminish the need for the adult to feed (Sheppard *et al*., 2002). In this study, three types of food waste (LBC, OR, & RB) were supplied in surplus and the performances of BSFL on the different feed substrates were compared. The results showed that all three feed substrates increased in length and weight of the BSFL, supporting the BSFL's growth. According to the USDA, rice grains have 80% carbs and 7% proteins per 100 g, whereas bananas have 23% carbohydrates and 1% proteins. The chicken's flesh is entirely composed of protein and has no carbohydrates. BSFL required a balanced diet to promote maximum growth and development and produce reproductively competitive adults (Gobbi *et al*., 2013). Cammack *et al*., 2017 and Jucker *et al*., 2017 suggest that a better-balanced ratio between protein and carbohydrate content may be responsible for the length and weight of BSFL larvae. The highest larval weight and length are seen in OR and RB, both of which contain all the nutrients required for BSL growth. However, because of an uneven diet (containing no carbohydrates), the LCB showed somewhat reduced length and weight. According to Barragan-Fonseca *et al*., 2019, dietary carbohydrate content has a greater impact on larval weight than dietary protein content.

RGR and BCR across different substrates were also examined in this work. The BCR demonstrated how effectively the BSFL transformed food waste into biomass, whereas the RGR revealed how quickly the BSFL developed on various substrates. Higher RGR and BCR values indicate favorable conditions for BSFL composting, whereas lower values signal environmental stress. OR and RB showed higher RGR and BCR values compared to LBC. This might be attributed to the poor quality of protein-based waste in LBC. According to Gobbi *et al*., 2013, the protein quality of the substrate could result in the slower development of BSFL. The amount and quality of protein components in the diet appear to be the primary determinants of development time (Jucker *et al*., 2017). In addition, the delayed development of BSFL fed on LBC waste could be linked to the presence of grease in the substrate, which makes it difficult for BSFL to digest the substrate, causing them to take longer to attain its ideal length and size (Barry, 2004).

SR and WRI were greatly influenced by the rearing substrate (Pliantiangtam *et al*., 2021). An appropriate rearing substrate could be used to achieve the highest decomposition efficiency and good-quality end products. All the investigated substrates in the current study showed greater SR and WRI values compared to the control, indicating improved composting activity with the presence of BSFL. Overall, BSFL was an excellent bio converter for carbohydrate-based waste (OR), followed by the fruit-based with carbohydrate and protein content of RB. The LBC waste that is categorized as protein-based waste was the least favored by BSFL. However, further investigation into other potential sources of uncertainty, such as the presence of grease, etc. is required to produce more conclusive results. In general, the results of this present study showed that the protein and carbohydrate composition of the feed substrates affect larval growth and development. In addition to BSFL's potential as a waste-valuation agent, the high feed conversion efficiencies and growth rates of these proteinaceous larvae make them an attractive and potentially lucrative feedstuff for livestock (Barragan-Fonseca *et al*., 2017). Using BSF, a variety of organic wastes have been valorized, with the by-product being an excellent nutrient source for animal feed (Veldkamp *et al*., 2012). Besides, it has been reported that research into the use of BSF larvae as a functional ingredient in pet food has advanced rapidly.

CONCLUSION

BSFL can be a useful tool for reducing food waste. The OR, RB, and LBC waste were successfully valorized within 15 days of the trials. In conclusion, the study demonstrated that all the substrates can be suitable feeds for the BSFL. The study found that larval development performance in terms of larval weight and length varied with the type of the substrates OR being the best performer followed by RB and LBC. Additionally, compared to LBC, the RB and OR substrates demonstrated a considerably greater growth rate and high capacity for converting biomass. OR and RB also showed the highest decomposition efficiency based on the SR and WRI value. Depending on local accessibility and nutrient needs, OR, RB, and LBC have the potential to be used for small and big-scale BSFL production.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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