

# Black Soldier Fly Larvae Growth, Nutritional Composition and Waste Reduction Performance on Food Waste with or Without Addition of Coconut Waste and Fermentation Process with Effective Microorganisms

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## ABSTRACT

The black soldier fly's (*Hermetia illucens*, Linnaeus 1758) larvae possess excellent potential for turning food waste into profitable products. Black soldier fly larvae (BSFL) need a diet high in protein to thrive but food waste diets in Malaysia are higher in carbohydrates than protein and contain high moisture, which can prevent the production of dry BSFL residue and highly nutritious larvae. Therefore, this research was conducted to improve food waste (FW) characteristics for BSFL development, waste reduction performance, and its nutritional composition by using the addition of coconut waste (CW) and through the fermentation process with effective microorganisms (EM). Overall, the FW50: CW50: EM10 group displayed the best BSFL growth development, followed by the FW100: EM10 and FW100 groups, and the FW50: CW50s group displayed the worst performance growth ( $p < 0.001$ ). The FW100: EM10 group had the highest waste reduction index (WRI), followed by FW100 and FW50: CW50: EM10, and the FW50: CW50s group had the lowest WRI ( $p < 0.001$ ). BSFL nutritional composition reared on diet group FW50: CW50: EM10 also has recorded the highest crude protein content, followed by FW100: EM10, FW50: CW50, and FW100. Therefore, compared to fresh food waste, a mixture of 50% food waste and 50% coconut waste, fermented with 10% EM (FW50: CW50: EM10) is probably the optimal mixture of rearing substrate for BSFL growth, best nutritional content, and waste reduction effectiveness. However, BSFL residue from this experimental group had a little higher moisture level than mature compost, so it needs to be cured or dried to reduce its moisture content.

**Key words:** Black soldier fly larvae growth, coconut waste, effective microorganisms, fermentation, food waste, waste reduction

## INTRODUCTION

Food waste is a major worldwide problem that contributes to both environmental degradation and food insecurity. Approximately 1.3 billion tonnes, or one-third, of all food produced for human use are wasted annually (FAO, 2013). Similar worrying statistics apply to Malaysia, where an estimated 0.9 kilogram of food waste is produced daily per person (Saeed *et al.*, 2009). This waste not only squanders valuable resources but also produces significant greenhouse gas emissions when decomposed in landfills (EPA, 2021). It is essential to address food waste using efficient treatment techniques to promote resource recovery and transform trash into useful goods. Efficient treatment for food waste can also help lessen their negative effects on the environment and also help to build a more sustainable and circular economy (Wang *et al.*, 2021).

There are a lot of drawbacks to traditional food waste treatment techniques including landfilling and incineration. Food waste from landfills builds up and breaks down anaerobically, releasing methane, one of the main greenhouse gases that causes climate change (EPA, 2021). Incineration, while reducing waste volume, can release harmful pollutants into the atmosphere and fail to recover any nutrients or energy from the waste (Abubakar *et al.*, 2022). These methods are less sustainable since they frequently fail to consider the possibility of resource recovery. Finding alternative food waste treatment such as composting using black soldier fly larvae (BSFL) is crucial for a more sustainable food waste management system. BSFLs not only efficiently biodegrade organic waste, but they also may convert it into high-quality protein and fat, which can be used in animal feed (Basri *et al.*, 2022). This method minimizes environmental impact, enhances nutrient recovery, and promotes a circular economy,

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addressing both waste management and food security challenges (Amrul *et al.*, 2022).

Black soldier flies (*Hermetia illucens*, BSFL) larvae were first discovered by Linnaeus in 1758. The morphological and physiological characteristics of BSFL allow them to absorb various organic sources such as food waste, agricultural waste, animal waste, and human waste (Gao *et al.*, 2019; Lim *et al.*, 2019; Liu *et al.*, 2020). One of the main benefits of BSFL in organic and food waste management is its capability to reduce organic waste to half of its initial weight, particularly during the larval period- a typical characteristic of a voracious feeder (Sarpong *et al.*, 2019). After the larvae phase, BSFL may reach their maximum size in the prepupae stage and abandon their food source in search of a drier and shady location to begin their pupation phase (also known as "self-harvesting") and the benefit from it is the minimization of the need for the previous labor-intensive stages of insect farming (Sheppard *et al.*, 1994; Amrul *et al.*, 2022). In terms of proximate composition, BSFL is high in protein (31–48%) and fat (26–33%) content which makes them more valuable lipids to use in animal feed production (; Amrul *et al.*, 2022; Meneguzet *al.*). Using BSFL to convert organic waste products into insect biomass is a feasible alternative method for sustainable waste management and an up-and-coming solution for sustaining circular economies that do not generate refuse and require fewer material and energy resources (Basri *et al.*, 2022a).

In Malaysia, the main problems in treating fresh food waste using BSFL are the high moisture content (>80%) and high-carbohydrate (>50%) but low-protein (<35%) contents of food waste (Chua *et al.*, 2019): The high moisture content of food waste (about 80%) provides a cooler temperature for the BSFL to live in; therefore, BSFL is more than likely to move out of the treatment container and leave the food waste untreated (Lalander *et al.*, 2020). Since BSFL prefers protein meals over carbohydrates for their growth (Jalil *et al.*, 2021), it is essential to improve the food waste content and nutrients before feeding it to BSFL. Improving the quality of food waste can be accomplished by incorporating other low-cost organic waste such as coconut waste (dregs), which can be added to control or reduce moisture content and support insufficient protein and fat nutrients in food waste.

Coconut is one of Malaysia's most popular fruits, with approximately 611 million coconuts consumed each year (Lim *et al.*, 2021). Particularly in Malaysian cuisine, coconut is a versatile and essential ingredient such as coconut milk and grated coconut flesh. Coconut milk is a basic ingredient that adds rich flavor and smoothness to many traditional meals, including curries and *nasi lemak* while grated coconut flesh is frequently used in snacks, sweets, and even savory recipes (Othman *et al.*, 2019). Due to its high consumption, consequently, it generates a large amount of coconut waste, especially coconut dregs. Other than that, coconut dregs are cheap and readily available in Malaysia, indicating that they are a potential source of continuous feed for BSFL. Therefore, this study aimed to use coconut dregs not only as additional protein in BSFL substrate but can be aimed to act as moisture control in the feed as it has high moisture absorption properties.

Nonetheless, according to a previous study (Basri *et al.*, 2022a), BSFL reared on only coconut dregs grow much slower than BSFL reared in self-fermented food waste due to the high lignocellulose content of coconut dregs (Raksasat *et al.*, 2020), which makes it difficult for BSFL to consume. As a result, we have seen the significance of the fermentation process in contributing to the growth of BSFL by improving raw material properties and increasing digestibility. BSFL prefer food (substrates) with easier digestibility primarily because it allows them to maximize their growth and development efficiently. Substrates with readily available nutrients such as proteins and fats are also suitable for BSFL because these nutrients are more easily assimilated in the larvae body mass (Amrul *et al.*, 2022). Additionally, fermentation changes the structure and size of substrate particles, resulting in a higher binding capacity to other ingredients when formulated in feed (Supriyati *et al.*, 2015; Andriani *et al.*, 2020).

Activated EM 1 or effective microorganisms activated solution (EMAS) is based on EM 1 which is the original and authentic effective microorganism product formulated by Professor Teruo Higa in 1982. EM 1 is an all-natural probiotic in a liquid form that contains various microorganisms such as lactic acid bacteria, yeast, and phototrophic bacteria (Higa & Parr, 1994). EM 1 and the metabolites it contains can activate natural microorganisms in a variety of settings and situations, as well as in a variety of fields like agriculture, animal husbandry, environmental purification, and healthcare, because these microorganisms coexist. To maintain the balance of microorganisms, activated EM 1 or EMAS is an extended form of EM 1. When molasses is used as a feed, the bacteria in EM 1 become more active and multiply. It can be expanded roughly 20 times relative to volume, but just once. EMAS can be combined with nearly any type of organic matter to facilitate the fermentation of organic materials (Higa & Parr, 1994). A study of the effects of fermentation utilizing EM, molasses, and urea on the nutritional composition of banana leaves revealed a significant increase in crude protein content and concluded that fermentation with EM is an appropriate pre-treatment method to improve food waste nutritional composition (Mat *et al.*, 2020). Therefore, this research aims to assess the development of BSFL growth, nutritional composition as well as its performance on waste reduction when reared on food waste with or without the addition of coconut waste and fermentation process with effective microorganisms using EMAS.

## MATERIALS AND METHODS

### Black soldier fly larvae

BSFL eggs were obtained by the BSFL farming industries, ZK Grenato Sdn. Bhd. BSFL eggs were hatched after four days and were reared on a pre-feed starter broiler chicken diet from Ayamas Integrated Poultry Sdn. Bhd. with nutritional composition of 21% crude protein, 5% crude fiber, 3.5% crude fat, 13% moisture, 7.5% ash, 0.7–1.4% calcium and 0.4–1.0% phosphorus. Initially, 500 young larvae (4–6 days old) were manually counted and separated from the pre-feed. The larvae were then gathered by average weight and randomly divided into four experimental groups, each with three repetitions (each duplicate weighing 1.5 g, or around 500 larvae). Each experimental group's larvae received four different diets to consume - 100% fresh food waste (FW100), a mixture of 50% fresh food waste and 50% coconut waste (FW50:CW50), 100% food waste fermented with 10% EM (FW100:EM10), and mixed of 50% food waste and 50% of coconut waste fermented with 10% EM (FW50:CW50:EM10).

### Rearing substrates

Food wastes were collected from Universiti Kebangsaan Malaysia dormitory cafeterias while coconut waste (dregs) was obtained from the small shop around Kajang, Selangor, Malaysia. As an early pre-treatment to avoid high food moisture content,

the food waste collected was sieved to ensure that its moisture content did not exceed 80%. The food waste was then blended using an Orimas QS505A Universal Fritter food processor. EM 1 and molasses were obtained from EMRO Malaysia Sdn. Bhd to produce an effective microorganism-activated solution in this study. EM 1 used in this study has active ingredients. This active ingredient refers to specific microorganisms contained in the EM 1 such as lactic acid bacteria, yeast, and phototrophic bacteria with a minimum of 1 million colony-forming units/cc (units/mL) and inactive ingredients of 99.99% of water and sugarcane (Higa & Parr, 1994; EMRO Malaysia, 2012). The preparation of EMAS followed according to the standard procedure from EMRO Malaysia Sdn Bhd (EMRO Malaysia, 2012) using the EMAS kit containing one liter of EM 1 and one liter of molasses. The first step was mixing 20-25 mL of molasses with 400 mL of clean water, adding 20-25 mL of EM 1 into the mixture, and stirring well. The mixture was poured into a 500 mL plastic bottle using a funnel, leaving a 4-5 cm air space at the top. The bottle was tightly capped and left in a warm place with an ideal fermentation temperature of 20-40°C. The cap was slowly loosened after a couple of days to release the gas and prevent overflow of the solution. The bottle was left to sit for another 1-2 weeks before use. The EMAS solution was ready for use when it produced a sweet-sour scent, and its color changed from black to reddish brown. The blended food waste was then mixed with or without coconut waste and EMAS. The EMAS was added to diets with a ratio of 1:10 w/v (EMRO Malaysia, 2012). For experimental groups with the fermentation process, the mixed substrate was left for anaerobic fermentation in a tightly closed and clamped container for 7 days before first feeding and was continuously left to undergo the fermentation process until the experiment ended. Table 1 shows the content and nutritional composition of experimental diets as determined by proximate analysis using the AOAC 20<sup>th</sup> Edition and Promerance Food Analysis: Theory and Practice, 2<sup>nd</sup> Edition.

**Table 1.** Content and nutritional composition of experimental diets

Index	Experimental Groups					
	FW100	FW50:CW50	FW100:EM10	FW50:CW50:EM10		
Fermentation with Effective Microorganisms	No	No	Yes	Yes		
Ingredients (% as fed)						
Food waste	100	50	100	50		
Coconut Waste	-	50	-	50		
Effective Microorganisms (EMAS)	-	-	10	10		
Analyzed nutritional composition (%Dry matter)						
Fermentation with Effective Microorganisms			Before	After	Before	After
Dry matter (%Fresh matter)	29.0	36.7	30.2	28.6	32.4	28.3
Ash	1.4	2.2	2.0	2.4	3.1	3.9
Crude protein	17.6	11.7	13.6	10.8	12.7	15.9
Total Fat	2.4	21.8	5.6	5.6	34.9	39.2
Crude fibre	-	19.3	-	-	26.2	32.9
Carbohydrate	78.6	64.3	78.8	81.1	49.4	41.0

#### Larval growth and waste reduction index

The research study was carried out at the Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia. The larvae composting experiments were conducted in 3000 mL square plastic containers. We cut the center of the square plastic container and fitted a mosquito net and a piece of thin cloth in its place to prevent the BSFL from crawling out of the containers and predators, such as ants, house flies, reptiles, and birds, from entering them. We tested each substrate in triplicates and fed 500 larvae every third day with a feeding rate of 60 mg/larva/day during the 12 days of the experiment. We added 90 grams of the substrate every third day (a total of 360 g of the substrate was added to each treatment container). The experiment was extended for six days to allow the BSFL to eat the leftover food before reaching the prepupae stage. Based on the researchers' previous laboratory trials, it is essential to extend the experiments to ensure there is no uneaten or excess feed in the treatment containers. The larval growth performance was evaluated by weighing and recording the weight of 20 larvae from each replicate every three days using an analytical balance (AND Weighing GR-200). At the point that forty percent of the larvae were at the prepupal stage, each replication was put to a stop (Nguyen *et al.*, 2015; Gao *et al.*, 2019). The diet and residue physiochemical parameters (temperature, pH, and moisture content) were all recorded and analyzed. For further analysis, larvae and prepupae at the end of the experiment were stored in a freezer at -20°C (Pliantiangtam *et al.*, 2021). Based on proximate analysis (AOAC 20<sup>th</sup> Edition & Promerance Food Analysis: Theory & Practice, 2<sup>nd</sup> Edition), the nutritional composition of larvae and prepupae was assessed. To determine the amount of waste consumed by the larvae and the efficiency of the substrate being converted into rewarding biomass, the waste reduction index (WRI) and substrate conversion efficiency were determined. According to Meneguz *et al.*, the formula for calculating substrate reduction (%SR), waste reduction index (WRI), and efficiency of conversion of digested food (ECD) was as follows:

$$\%SR = \frac{\text{Distributed substrate (g)} - \text{Residual substrate (g)}}{\text{Distributed substrate}} \times 100$$

$$WRI = \left[ \frac{\text{Distributed substrate (g)} - \text{Residual substrate (g)}}{\text{Distributed substrate (g)}} \right] \times 100 \div \text{Days of trial (day)}$$

$$ECD = \frac{\text{Larval and prepupae weight (g)}}{\text{Distributed substrate (g)} - \text{Residual substrate (g)}}$$

### Statistical analysis

This study was conducted using a fully randomized approach. One-way analysis of variance (ANOVA) was performed using Duncan's multiple range test as a post-hoc analysis to evaluate the differences in all measured, analyzed, and calculated data between experimental groups (fixed factors). Both Levene's test and the Shapiro-Wilk test have confirmed the homogeneity of variance and the normal distribution. A difference that was recognized as statistically significant was  $p < 0.05$ . IBM SPSS Statistics 20 was used to conduct all statistical analyses in the study.

## RESULTS

Table 2 represents the growth performance and waste reduction efficiency reared on food waste with or without the addition of coconut waste and fermentation process with effective microorganisms. Following the FW100:EM10 and FW100 groups, the FW50:CW50:EM10 group had the highest final larval and prepupal weight; the FW50:CW50s group had the lowest performance growth ( $p < 0.001$ ). The FW100:EM10 group had the highest %SR, followed by the FW100 group and FW50:CW50:EM10, and the FW50:CW50s group had the lowest %SR ( $p < 0.001$ ). The FW100:EM10 group had the highest WRI, followed by FW100 and FW50:CW50:EM10, and the FW50:CW50s group had the lowest WRI ( $p < 0.001$ ). In comparison to diet groups without EMAS fermentation (FW100 and FW50:CW50), all groups fed with EMAS fermentation had the highest ECD (FW50:CW50:EM10 & FW100:EM10). At the start of the experiment, the pH of the fermented group diets varied greatly from the unfermented diet: 3.5-3.8 and 5.2-5.5, respectively. The feed with the lowest pH at the start of the study was FW100:EM10, then FW50:CW50:EM10, FW50:CW50, and FW100, in that order. The pH value of the feeding substrate on harvesting day was observed higher than at the beginning for FW100, FW100:EM10, and FW50:CW50:EM10 groups respectively. The highest moisture of the beginning feeding substrate was obtained from the FW100 group, followed by FW50:CW50:EM10, and FW100:EM10; the lowest moisture of feed at the beginning was from the FW50:CW50s group ( $p < 0.001$ ). The moisture of feed at the end of the experiment was recorded as the lowest in the FW50:CW50 group, followed by FW100, FW50:CW50:EM10, and the highest were from the FW100:EM10s group ( $p < 0.001$ ). The temperature of feed at the beginning of the experiment was recorded as highest in the FW50:CW50 group followed by FW50:CW50:EM10, FW100:EM10, and FW100 group respectively ( $p < 0.001$ ). Following 18 days of treatment, the diet group FW50:CW50 had the highest temperature of BSFL residue, followed by FW50:CW50:EM10, FW100:EM10, and FW100.

The nutritional composition of BSFL-reared food waste with or without the addition of coconut waste and fermentation process with effective microorganisms is also presented in Table 2. The lowest BSFL dry matter between diet groups is FW100:EM10, followed by FW50:CW50:EM10, FW50:CW50, and FW100 groups. The highest ash in BSFL composition is when BSFL fed on the FW50:CW50 diet group, followed by FW100:EM10, FW50:CW50:EM10, and the lowest was observed in the FW100s group. The highest crude protein percentage was present in FW50:CW50:EM10, followed by FW100:EM10, FW50:CW50, and FW100. Meanwhile, the highest total fat percentage in BSFL was present in FW50:CW50, followed by FW100:EM10, and FW50:CW50:EM10; the lowest total fat percentage in BSFL was obtained when fed on the FW100s group. Crude fiber percentage in BSFL was only present in the FW100 group, and the highest carbohydrate percentage obtained from BSFL composition was from FW100 feedstock and  $< 0.3$  for the other three groups; FW50:CW50, FW100:EM10 and FW5:CW50:EM10 group, respectively.

## DISCUSSION

### Nutritional composition of rearing substrates

Development of BSFL such as growth and its nutritional composition is believed to be significantly influenced by the nutritional composition of rearing substrates (Pliantiantam *et al.*, 2021). Based on Table 1, the addition of coconut waste into food waste as in group FW50:CW50 has shown increasing dry matter content. Previous studies have shown that substrates with a 60-75% moisture content are ideal for BSFL rearing and facilitate the dry separation of BSFL from the residue (Sheppard *et al.*, 2002; Diener *et al.*, 2009; Cheng *et al.*, 2017). Substrates with less than 60% moisture content will hinder the development of BSFL as the larvae digest better with semi-solid substrates while substrates with greater than 75% moisture will lead to an anaerobic condition of the substrates, inactive composting process and produce wet frass (Basri *et al.*, 2022a). The moisture content of BSFL substrates or feedstock is crucial for the metabolic activity of the larvae and microbes because it indirectly provides oxygen (Bernal *et al.*, 2009; Dortmans, 2015). The water content of the feedstock in this study was sufficient for BSFL breakdown activity. As for the fermentation process with effective microorganisms using EMAS also has proven to increase protein content with the addition of coconut waste (12.7 to 15.9%) compared to the decrease of protein content in the fermentation of food waste without the addition of coconut waste (13.6 to 10.8%). The experimental group with additional coconut waste also showed the



highest total fat in chemical composition while the highest crude fiber composition from the experimental group was from the experimental group with the addition of coconut waste. The highest carbohydrate composition was present in the fermented food waste group, FM100:EM10 (81.1) followed by fresh food waste (78.6). The present chemical composition has shown that a mixture of food waste with additional coconut waste and has undergone a fermentation process with EMAS had increased protein, fat, and crude fiber composition. The BSFL fed with fermented showed the fastest growth than with unfermented group. This result matched the results from Somroo *et al.*, (2019), which showed that fermentation of soybean curd residue has a higher nutritional composition than unfermented soybean curd residue. While the BSFL fed on food waste with additional coconut waste in the FW50:CW50s group has shown the slowest larval growth. These results also have been proven by a previous study by Basri *et al.* (2022a), which shows low growth performance of BSFL; this is due to the high lignocellulose content of coconut waste (dregs), which was hard to consume by BSFL (Raksasat *et al.*, 2020).

**Table 2.** Nutritional composition of BSFL, growth performances, and waste reduction efficiency BSFL reared on food waste with or without the addition of coconut waste and fermentation process with effective microorganisms

Parameters <sup>1</sup>	Experimental groups				SEM	p-Value
Group	FW100	FW50:CW50	FW100:EM10	FW50:CW50:EM10		
Fermentation with Effective Microorganisms	No	No	Yes	Yes		
Ingredients (% as fed)						
Food waste	100	50	100	50		
Coconut Waste	-	50	-	50		
Effective Microorganisms (EMAS)	-	-	10	10		
Growth performances <sup>2</sup>						
Larval weight at day 0 of treatments (g)	0.06	0.05	0.07	0.06	0.002	0.251
Larval weight at day 3 of treatments (g)	0.88 <sup>a</sup>	0.86 <sup>a</sup>	0.85 <sup>a</sup>	0.89 <sup>a</sup>	0.012	0.539
Larval weight at day 6 of treatments (g)	1.06 <sup>c</sup>	1.42 <sup>b</sup>	1.17 <sup>c</sup>	1.92 <sup>a</sup>	0.102	<0.001
Larval weight at day 9 of treatments (g)	2.24 <sup>c</sup>	1.88 <sup>d</sup>	2.63 <sup>b</sup>	3.67 <sup>a</sup>	0.206	<0.001
Larval weight at day 12 of treatments (g)	2.45 <sup>c</sup>	2.61 <sup>c</sup>	3.75 <sup>b</sup>	4.12 <sup>a</sup>	0.217	<0.001
Larval weight at day 15 of treatments (g)	2.70 <sup>b</sup>	2.84 <sup>b</sup>	3.80 <sup>a</sup>	4.19 <sup>a</sup>	0.204	<0.001
Prepupae and larval weight at day 18 of treatments (g)*	2.57 <sup>c</sup>	2.53 <sup>c</sup>	3.78 <sup>b</sup>	4.28 <sup>a</sup>	0.231	<0.001
Waste reduction efficiency						
Substrate reduction (%)	92.7 <sup>b</sup>	87.3 <sup>d</sup>	99.0 <sup>a</sup>	89.3 <sup>c</sup>	1.345	<0.001
Waste reduction index (g/d)	5.14 <sup>b</sup>	4.86 <sup>d</sup>	5.51 <sup>a</sup>	4.97 <sup>c</sup>	0.075	<0.001
The efficiency of conversion of digested food (%)	0.19 <sup>c</sup>	0.20 <sup>c</sup>	0.26 <sup>b</sup>	0.33 <sup>a</sup>	0.017	<0.001
pH of feed						
Beginning of experiment	5.5 <sup>a</sup>	5.2 <sup>b</sup>	3.5 <sup>d</sup>	3.8 <sup>c</sup>	0.261	<0.001
Harvesting day	6.5 <sup>b</sup>	5.9 <sup>d</sup>	6.5 <sup>c</sup>	6.6 <sup>a</sup>	0.079	<0.001
moisture of feed (%)						
Beginning of experiment	76.0 <sup>a</sup>	68.0 <sup>d</sup>	70.6 <sup>c</sup>	72.7 <sup>b</sup>	0.896	<0.001
Harvesting day	35.4 <sup>c</sup>	24.8 <sup>d</sup>	62.1 <sup>a</sup>	48.6 <sup>b</sup>	4.221	<0.001
temperature of feed (°C)						
Beginning of experiment	32.2 <sup>c</sup>	34.4 <sup>a</sup>	32.4 <sup>c</sup>	33.4 <sup>b</sup>	0.269	<0.001
Harvesting day	27.0 <sup>b</sup>	27.5 <sup>a</sup>	27.1 <sup>b</sup>	27.2 <sup>b</sup>	0.701	0.005
Analyzed nutritional composition of BSFL (% Dry matter)*						
Dry matter (% Fresh matter)	38.2	36.3	33.1	35.5		
Ash	3.1	4.1	3.9	3.4		
Crude Protein	42.7	44.6	45.9	47.9		
Total fat	42.9	51.2	50.2	45.9		
Crude fibre	11.3	0.0	0.0	0.0		
Carbohydrate	7.6	<0.3	<0.3	<0.3		

<sup>1</sup> The differences on the same row's superscripts indicate the statistically significant difference at  $p < 0.05$ . <sup>2</sup> The growth performance of prepupae was based on fresh larval and prepupal weight in gram,  $n = 20$ . \*The analyzed nutritional composition of BSFL is based on one combined sample from three replicates of BSFL fed on different diets.

#### Black soldier fly larvae residue characteristics.

Temperature is the critical factor determining whether decomposition occurs in the mesophilic or thermophilic phase or achieves maturity to produce natural plant fertilizer (Kamaruzzaman *et al.*, 2018). After the degradation process from either conventional composting or insect bioconversion, humus materials that reach room temperature throughout the process have reached maturation (Attigbo *et al.*, 2019; Hamid *et al.*, 2019; Ho *et al.*, 2022). Based on Table 2, the two groups of mixed food waste and coconut dregs have the highest temperature of the substrate; 34.4°C for FW50:CW50 group and 33.4°C for FW50:CW50:EM10. Whereas the groups without the mix of coconut dregs show the lowest temperature of the substrate; 32.2°C for the FW100 group and 32.4°C for FW100:EM10. Subsequently, the two groups of mixed food waste and coconut dregs have

the highest temperature of residue; 27.5°C for FW50:CW50 group and 27.2°C for FW50:CW50:EM10. Whereas the groups without the mix of coconut dregs show the lowest temperature of the substrate; 27.0°C for the FW100 group and 27.1°C for FW100:EM10. This result shows that the addition of coconut dregs into food waste has increased the temperature of the BSFL composting process thus resulting in higher residue temperatures. According to previous research using food waste substrate, residue harvested from the BSFL bioconversion process has an average temperature of 24 – 27°C, this is because the BSFL composting process occurred in the mesophilic phase (Sarpong *et al.*, 2019; Attiogbe *et al.*, 2019; Pang *et al.*, 2020). The mesophilic phase in BSFL refers to a specific temperature range (20°C & 45°C) and microbial activity of bacteria and fungi proliferate decompose the food waste and making nutrients available for BSFL (Sarpong *et al.*, 2019; Attiogbe *et al.*, 2019; Basri *et al.*, 2022b).

It is essential to determine the pH of residue since it is a helpful parameter for tracking the efficiency of the decomposition process. A rising pH value in the decomposition phase indicates that the BSFL and microbial action has completed the generation of acid and ammonia and is related to compost curing (Sarpong *et al.*, 2019). Among the experimental groups, the groups with the fermented process had more acidic substrates (FW100:EM10 & FW50:CW50:EM10) than the groups without it (FW100 & FW50:CW50). This is because the fermented process occurred in anaerobic conditions while the groups without the fermentation process contained fresh organic waste. At the end of the BSFL composting process, the highest pH value for residues was from the FW50:CW50:EM10s group (6.6) followed by FW100 (6.5), FW100:EM10 (6.5) and FW50:CW50 (5.9). Generally, the pH of the residue from food waste substrates ranges from 5.6-8.0 (Basri *et al.*, 2022b), while the pH of mature compost ranges from 6.0-8.0 (El-Haggar, 2007). In this experiment, all experimental groups have approached the desired pH value for mature compost. With a pH range of 5.9 – 6.6. Previous research has demonstrated that BSFL can withstand acidic conditions (pH = 2-6) and change the substrate's pH value to the desired level (Meneguz *et al.*, 2018).

Studies have shown that the coconut waste from the coconut milk processing factory is an excellent adsorbent because of its high adsorption capacity, good regeneration capabilities, and low cost compared to commercial adsorbents (Rahim *et al.*, 2021). The residue for experimental group FW100 and FW50:CW50:EM10 had a moisture content of 35.4%, and 48.6% respectively. A mature compost can be identified when the moisture of the compost is within 30-45% (Basri *et al.*, 2022b). Since residue in the experimental group, FW50:CW50:EM10 has a slightly higher moisture content than the compost mature range, extending the curing or drying of the residue could reduce its moisture content.

#### *Black soldier fly larvae protein and fat composition.*

BSFL protein and fat composition from fresh food waste may range from 36-48% and ~35% respectively (; Lalander *et al.*, 2019; Gold *et al.*, 2020), while the BSFL protein composition from a mixture of coconut waste shows relatively low protein content and high-fat content; 22-40% and 39-48% respectively (Lim *et al.*, 2019; Muchdar *et al.*, 2021). On the other side, BSFL reared on fermented food waste was found to have 15-42% protein content and 31-58% fat content (Mohd-Noor *et al.*, 2017; Gao *et al.*, 2019). The results of this study show that the highest BSFL protein content is obtained from the FW50:CW50:EM10 feed media formulation which is 48% compared to the control feed media formulation which is FW100 (43%) and also FW50:CW50 (45%). This result is the same as the highest BSFL protein content from BSFL consumes domestic food waste which is 48% (Sprangers *et al.*, 2017). Meanwhile, the highest BSFL fat content of 51.2% was obtained from FW50:CW50 and the FW100:EM10 experimental group was the second highest at 50.2%. This result can be compared with the range of BSFL fat content that consumed coconut waste and fermented food waste which produce higher fat composition than protein in their biomass (Mohd-Noor *et al.*, 2017; Gao *et al.*, 2019; Lim *et al.*, 2019; Muchdar *et al.*, 2021). BSFL reared on FW50:CW50 also has shown the lowest growth, this is because this experimental group contains a fresh mix of food waste and coconut waste. Fresh coconut waste (dregs) used in this experiment was high in fiber which contains high lignocellulosic compounds. The lignocellulosic compound was hard to digest by BSFL thus providing not enough nutrients for larvae growth (Raksasat *et al.* 2020). Compared to the FW5:CW50:EM10 group, the coconut waste was fermented resulting in higher available nutrients for maximum BSFL growth. Gao *et al.* (2019) also reported that the fermentation of maize straw has improved the digestibility and palatability of larvae. Overall, BSFL nutritional content reared in the FW50:CW50:EM10 experimental group contains balanced protein (48%) and fat (46%) content, making it suitable to be used as an alternative protein source for animal feed, biodiesel production, and so on. Therefore, based on the results of this study, the best feed media formulation for the acquisition of black soldier fly larval composting products is a mixture of 50% food waste and 50% coconut waste fermented with 10% EMAS probiotics (FW50:CW50:EM10).

#### *EM strains impact on BSFL.*

In this experiment, the EM strains used in the FW100:EM10 group and FW50:CW50:EM10 group are a combination of lactic acid bacteria, yeast, and phototrophic bacteria. By referring to Table 2, larvae from both groups have shown the highest protein content (45.9%, 47.9%) and the highest efficiency of conversion of digested food (0.26%, 0.33%). The study's findings were consistent with other research that found EM affects BSFL composting. Application of beneficial effective microorganisms such as *Lactobacillus buchneri* had significantly contributed to higher dry mass reduction (56%), bioconversion rate (7%), crude protein content (55%), and fat content (30%) than fed on untreated soybean curd residue and artificial feed (Somroo *et al.*, 2019). Research on *in-situ* yeast fermentation has been done to increase protein levels in feed with low protein content and rich in starch such as coconut waste showed an increase in lipid yield and lipid productivity of BSFL by 49.4% and 0.53 g/day, respectively (Wong *et al.*, 2020).

## CONCLUSION

In this study, our aim to improve the nutrition of food waste with the addition of coconut waste and fermentation with probiotics EMAS has shown satisfying results on BSFL development through its growth, nutritional composition as well as waste reduction performance. The FW50:CW50:EM10 experimental group's BSFL demonstrates the possibility of this diet yielding dry residue and very nutritious prepupae. However, it is necessary to cure or dry the BSFL residue to lower its moisture content because the

moisture level of the residue from this experimental group was marginally greater than that of mature compost. Other than that, there were some challenges observed during larval growth, especially in the FW50:CW50 group where the larval growth was the lowest. This happens because fresh coconut dregs contain a lot of lignin, which is difficult for BSFL to digest, there aren't enough nutrients for the larvae to thrive. From the result of this study, the highest larval growth is from the FW50:CW50:EM10 group. Therefore, to boost BSFL substrate nutritional content, coconut dregs or lignin-rich substrates must be fermented.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ETHICAL STATEMENT

Not applicable.

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