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Fermented black soldier fly (*Hermetia illucens*) carcass meal as a substrate for its larva

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Abstract. *Riok PAH, Hariani N, Nugroho RA, Arif MF, Syafrizal, Aryani R, Manurung H, Rudianto. 2025. Fermented black soldier fly (*Hermetia illucens*) carcass meal as a substrate for its larva. Nusantara Bioscience 17: 49-56.* The black soldier fly (*Hermetia illucens* (Linnaeus, 1758)) is an insect commonly used as an organic waste decomposer. The Black Soldier Fly (BSF) has four life stages, and as an adult, it dies shortly after reproducing, which leads to it being an underutilized waste product. This study aimed to determine and compare the effects of the fermented BSF Carcass Meal (fBSFM) and Fermented Palm Kernel Meal (fPKM) as substrates on the growth rate and proximate composition of BSF Larvae (BSFL). The present research used a completely randomized design with four groups: a control group (100% fPKM) and three experimental groups with varying percentages of fBSFM (P1: 5% fBSFM, P2: 7.5% fBSFM, P3: 10% fBSFM). The results showed that substituting fBSFM at concentrations of 7.5- and 10% in fPKM increased growth rate, individual weight, biomass, and bioconversion, supporting their life cycle; the use of 15% fBSFM as BSFL substrate significantly impacted substrate reduction. The substrate reduction rate was measured at 55.18 ± 8.49%, suggesting that black soldier fly larvae can effectively reduce both fBSFM and fPKM. The different proportions of fPKM and fBSFM did not have a significant effect on the proximate values of the BSFL. Therefore, this research provides valuable insight for waste management and agriculture, suggesting that incorporating more than 5% of fermented BSF carcass meal into the substrate mixture with fPKM is advisable to enhance the growth indices of BSFL.

Keywords: Black soldier fly meal, *Hermetia illucens,* larval growth, proximate composition

INTRODUCTION

The Black Soldier Fly (BSF) was officially recognized as *Hermetia illucens* (Linnaeus, 1758) (Diptera: Stratiomyidae), has numerous nutritive value benefits for cattle and aquaculture (Rehman et al. 2023; Ferdousi et al. 2024). The BSF is commonly located in humid, nutrient-dense environments, marked by a significant accumulation of decaying organic matter from animals and plants (Diola et al. 2024; Lomonaco et al. 2024; Septiariva et al. 2024). The BSF Larvae (BSFL) possess several advantageous characteristics, such as a comparatively high protein and lipid value, the capacity to thrive on diverse waste materials unsuitable for human consumption, and their non-classification as a pest or nuisance species (Liu et al. 2022; Phaengphairee et al. 2023). The nutritional profile of BSF larvae can comprise as much as 40% protein (Ndotono et al. 2022) and 30% lipid (Lin et al. 2022), with variations contingent upon the substrate used for their growth (Newton et al. 2005; St-Hilaire et al. 2007). Various kinds of organic substrates influence the production and nutritive quality of BSFL (Lalander et al. 2019; Truzzi et al. 2020; Fischer and Romano 2021). The BSFL typically demonstrates accelerated development when provided with a diet containing a balanced ratio of easily digestible protein and carbohydrates (Cammack and Tomberlin 2017). Nevertheless, substrates with elevated protein levels may further promote larval growth, resulting in larger larvae (Nguyen et al. 2015; Tinder et al. 2017; Lalander et al. 2019).

The life cycle of BSF can be distinguished into four main steps: egg, larva, pupa, and adult. In larval steps, the tiny BSFL begin to feed on the organic matter around them after hatching. They are equipped with powerful mandibles that allow them to shred and consume various materials. As they feed, the larvae grow rapidly, shedding their exoskeletons several times as they molt. The larval step can last anywhere from 10 days to several weeks, depending on the availability of food and environmental conditions (Oliveira et al. 2016). Once they have reached their maximum size, the larvae enter a non-feeding stage called the prepupal stage, during which they seek out a dry, sheltered location to begin their transformation into pupae. During the pupal stage, the larvae transform into a non-feeding, immobile pupa. The pupal stage typically lasts about 1-2 weeks, during which time the pupa undergoes metamorphic changes to emerge as an adult fly (Muhayyat et al. 2016).

Finally, the adult BSF is a stout, shiny black insect with a distinctive pattern of white markings on its abdomen. Adults are strong fliers and are attracted to light. They feed on nectar, fruit juices, and other sweet substances. Males and females mate soon after emergence, and the female begins laying eggs within a few days. The adult stage typically lasts about 4-6 weeks, depending on environmental conditions. After mating, the male BSF died, followed by the female BSF soon after laying their eggs (Wardhana 2016). The dead body or carcass of both male and female BSF is usually thrown away and no longer used in the BSF farm. However, it's important to note the ecological significance of the adult BSF. Despite their short lifespan, these insects play a crucial role in waste recycling and resource recovery, particularly when integrated with palm oil plantation by-products.

Moreover, biomass that contains palm fronds and trunks is generated as a result of replanting operations in oil palm plantations. Palm oil mills produce various types of by-product waste while converting Fresh Fruit Bunches (FFB) into Crude Palm Oil (CPO). These include Empty Fruit Bunches (EFB), Mesocarp Fiber (MF), Palm Kernel Shell (PKS), Palm Kernel Meal (PKM), and Palm Oil Mill Effluent (POME). Indonesia has a significant abundance of these wastes, found in various locations across 22 provinces, including oil palm fields, FFB manufacture, and palm oil mills. These waste products provide economic worth as they may be converted into alternative fuels, fertilizers, and biochemical materials. Therefore, a pioneering study is essential to augment the value of PKM and foster sustainability in the oil palm sector (Hambali and Rivai 2017). Azizi et al. (2021) discovered that Palm Kernel Cake (PKC) has a Crude Protein (CP) content of around 14-18%, Crude Fiber (CF) content of 12-20%, Ether Extract (EE) content of 3-9%, and other minerals. Furthermore, Balandrán-Quintana et al. (2019) reported that PKM has a protein value between 14.4 and 20%, along with significant quantities of carbohydrates (50.3%) and crude fiber (16.7%).

Several studies have examined the growth of BSFL in various substrates (Spranghers et al. 2017; Shumo et al. 2019; Nugroho et al. 2024). However, no study has investigated the growth and nutritive value of BSFL raised in fermented Black Soldier Fly Carcass Meal (fBSFM) and fermented PKM (fPKM). This study aimed to examine the development and nutritional composition of the BSFL grown in a substrate containing fBSFM compared to fPKM. It represents the first report on the use of BSF carcasses as a potential substrate for their larvae.

MATERIALS AND METHODS

Study area

The present study was carried out at the laboratory of Animal Physiology, Development, and Molecular, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Mulawarman in Samarinda, East Kalimantan, Indonesia.

BSFL meal preparation

The BSF carcasses were dried using a dehydrator for 48 hours. The dried BSF carcass was ground into a meal using a food processor. The BSF meal (BSFM) was fermented for 5 days before being used as BSFL substrate. The fermented substrate was evaluated for proximate analysis. Table 1 shows a formula to make a fermented substrate for BSFL.

BSFL preparation and acclimatization

The BSFLs were reared from the egg phase, which was obtained from CV. Ahasa Larva Group, Samarinda, East Kalimantan, Indonesia. The eggs of BSF were randomly placed in four plastic boxes (35 length × 25 width × 12 height cm). Each box was filled with 3.25 g of BSF eggs. First instar BSFL hatchlings were given a mixture of fish pellets and water (100 g: 1 liter), 325 g/3 days for 7 days. The instar of BSFL was reared in a condition of temperature 27-29°C and humidity 60-80%. During the 1st instar of the BSFL rearing process, the substrate was sprayed with water daily to keep the substrate and larvae moist.

BSFL for proximate analysis

In the treatment stage, the BSFL rearing was divided into two purposes: proximate and growth analysis. For proximate analysis, an amount of 28.25 g of BSFL with an estimated number of ± 2,500 of seven seven-Day-Old Larvae (DOL) was put into the rearing tanks. Subsequently, the formulated fermented substrate was given for 10 days. Meanwhile, for growth analysis, a number of 30 individual larvae were weighed and placed into the rearing container (500 mL plastic bottle). The BSFL was given various fermented substrates for 10 days. Both BSFL for proximate and growth analysis were fed with fermented substrates following a previous study by Guo et al. (2021). All treatments of BSFL, either for proximate or growth analysis, had three replications. All plastic boxes and containers were placed in the room with a temperature of 27-29°C and humidity of 60-80% condition. On the 11th day, all larva was harvested for proximate and growth analysis. The harvested BSFL for proximate analysis was dried using a microwave. The dried BSFL was ground using a food processor to obtain a BSFL meal. The BSFL meal was weighed and stored in the freezer until it was used for proximate analysis.

Table 1. Black Soldier Fly Larvae (BSFL) substrate fermentation formula

Groups	Ingredient (g) (PKM:BSFM)	Molasses (g)	EM4 (mL)	Water (L)
fPKM	1000: 0	40	62	2
fBSFM 5%	950: 50	40	62	2
fBSFM 10%	925: 75	40	62	2
fBSFM 15%	900: 100	40	62	2

Note: fPKM: 100% Fermented Palm Kernel Meal; fPKM: various ratios of Palm Kernel Meal (PKM) with Black Soldier Fly Meal (BSFM). The addition of EM4 and water are based on the previous study (Nugroho et al. 2024; Nugroho et al. 2023)

Growth parameter analysis

The individual weight of the BSFL from the control and each treatment group was performed using an analytical balance (220 g × 0.0001 g). The individual weight of the larvae was calculated using the following formula (Romano 2022):

$$\text{Individual weight} = \text{Final individual weight} - \text{initial individual weight}$$

Biomass measurement was measured from either control or treatment groups of the BSFL fed various fermented substrates using an analytical balance and followed the previously used equation (Romano 2022):

$$\text{Biomass} = \text{Final population weight} - \text{initial population weight}$$

The growth rate of the BSFL was determined by dividing the average weight of an individual by the total treatment time. The growth rate was calculated using the following equation (Wevers et al. 2022):

$$GR = \frac{(W_f - W_i)}{T}$$

Where: GR: Growth Rate (gr/day); Wf: Average final weight of individuals BSFL (g); Wi: Average initial weight of individuals (g); T: Duration of the study (10 days).

Substrate Reduction (SR)

Substrate Reduction (SR) aims to determine the efficiency of the BSFL in reducing the fermented substrate or substrate given. The SR value was calculated by dividing the total weight of the fermented substrate consumed by the BSFL by the total weight of the fermented substrate. The SR was determined using the following formula (Diener et al. 2009):

$$SR = \frac{(FS_t - FS_r)}{FS_t} \times 100\%$$

Where: SR: Substrate Reduction (%); FS_t: Total weight of fermented substrate (54.2 g); FS_r: Weight of remaining fermented substrate (g).

Substrate bioconversion or Biomass Conversion Ratio (BCR) is the efficiency value of the BSFL in bioconverting fermented substrate into useful biomass. The BCR can be determined by dividing the BSFL biomass value by the total weight of the fermented substrate given. The BCR was evaluated following the equation used by Lindberg et al. (2022):

$$BCR = \frac{(\text{BSFL biomass final weight} - \text{BSFL biomass initial weight})}{FS_t} \times 100\%$$

Where: BCR: Bioconversion of Ratio (%); FS_t: Total weight of fermented substrate (54.2 g).

Survival

The BSFL survival percentage was determined by dividing the number of survival BSFL at the end of the study by the total BSFL population at the beginning of the study.

Proximate analysis

Proximate analysis was evaluated on the fermented substrate sample and black soldier fly larva meal. Each group of the BSFL was then processed for proximate evaluation, which included the assessment of crude protein, crude lipid/fat, fiber, ash, and moisture value. Crude protein analysis was conducted using the standard technique of the Association of Official Analytical Chemists (AOAC), AOAC International, 17th ed v.2, Gaithersburg, Md method (Horwitz 2000). Moisture, ash, and crude fiber content were determined using the gravimetric method as described by BSN (1992). Crude fat content was determined using BSN (1992) method, using the soxhlet extraction method. The proximate parameters content was determined following equations:

Crude protein

$$\%N = \frac{[(N_{acid})(mL_{acid}) - (mL_{bk})(N_{NaOH}) - (mL_{NaOH})(N_{NaOH})][1400{,}67]}{\text{mg sample}}$$

$$\%\ \text{Protein} = \%\,N \times \text{Conversion factor}$$

Where: mL_{NaOH}: mL standard base needed to titrate sample; $mL_{acid:}$ mL standard acid used for sample; $mL_{bk:}$ mL standard base needed to titrate reagent blank; N_{acid}: Acid normality; N_{base}: Base normality; conversion factor: 6.25.

Crude fat

$$\%\ \text{Crude fat} = \frac{W_1 - W_2}{W_0} \times 100\%$$

Where: W_0: Sample weight (g); W_1: Boiling flask weight (g); W_2: Boiling flask + fat weight (g).

Moisture content

$$\%\ \text{Moisture} = \frac{W_1 - W_2}{W_0} \times 100\%$$

Where: W_0: Sample weight (g); W_1: Crucible + sample weight before drying (g); W_2: Crucible + sample weight after drying (g).

Ash content

$$\%\ \text{Ash} = \frac{W_1 - W_2}{W_0} \times 100\%$$

Where: W_0: Sample weight (g); W_1: Crucible + ash sample weight (g); W_2: Crucible weight (g).

Crude fiber

$$\% \text{ Crude fiber} = \frac{W_1 - W_2}{W_0} \times 100\%$$

Where: W_0: Sample weight (g); W_1: Filter paper + fiber residue (g); W_2: Filter paper weight (g).

Data analysis

The data collected was analyzed utilizing SPSS version 25 (SPSS, Inc., USA). The Levene test was used to evaluate the normality of the acquired data. The statistical test results stated that the growth parameters data (individual weight, biomass, bioconversion, and growth rate) were normally distributed and homogeneous; the normally distributed and homogeneous data were analyzed using One Way ANOVA followed by Duncan's test with a 95% confidence level to determine the significance. Data that were not normally distributed and not homogeneous (substrate reduction, mortality, crude protein, crude fat, crude fiber, ash, and moisture) were analyzed with the Kruskal-Wallis non-parametric test.

RESULTS AND DISCUSSION

Based on the proximate analysis of various fermented substrates, it is shown that the fermentation process of BSFM, in combination with PKM, resulted in various proximate values (Table 2**)**. The protein content increases significantly by adding BSFM to the Palm Kernel Meal (PKM). The highest protein content was observed in the fBSFM 15% substrate, indicating that increasing the proportion of BSFM enhances the protein content. Fat content was relatively stable across all substrates, with only minor variations. This shows that the fermentation process has no real effect on fat content. Water content decreased as the proportion of BSFM increased. The lower water content in BSFM may cause this compared to PKM. Similar to moisture content, the ash content decreases slightly with the addition of BSFM. This might indicate a reduction in mineral content or changes in the mineral composition due to fermentation. Further, the fiber content slightly increases with higher proportions of BSFM. This could be due to the fibrous nature of BSFM.

As Table 3 shows, different ratio concentrations of fBSFM and fPKM varied the growth parameters values of BSFL. The fPKM group has the lowest individual weight (0.1195 ± 0.0012 g). Any levels of fBSFM improved individual weight and growth rate of BSFL compared to BSFL reared with fPKM. Various levels of the fBSFM 5-15% substrate groups significantly affected bioconversion and growth rate. The use of fBSFM higher than 5% enhanced the biomass of the BSFL. The highest percentage of substrate reduction (55.18 ± 8.49%) was found on the BSFL grown on fBSFM 15%. There was no significant difference in the BSFL survival which was reared using any substrate.

Various substrates given for BSFL did not significantly affect (*P>0.05*) all proximate parameters, including crude protein and crude fiber content. However, crude fat, moisture, and ash content may be considered significant based on the percentage form, which indicates a magnitude of more than 5% (Table 4).

Table 2. Proximate value of the various fermented substrates of the black soldier fly larvae

Parameters (%)	Groups			
	fPKM	**fBSFM 5%**	**fBSFM 10%**	**fBSFM 15%**
Crude protein	6.33	20.74	21.42	22.15
Crude fat	7.86	7.13	7.27	7.36
Moisture	33.14	13.81	13.56	13.24
Ash	5.27	4.36	4.51	4.70
Crude fiber	12.93	12.92	13.05	13.38

Noted: fPKM: 100% Fermented Palm Kernel Meal; fPKM: Fermented Various Ratios of Palm Kernel Meal (PKM) with Black Soldier Fly Meal (BSFM)

Table 3. The growth rate average value of BSFL fed various combinations of fermented black soldier fly meal and fermented palm kernel meal

Parameters	Groups			
	fPKM	**fBSFM 5%**	**fBSFM 10%**	**fBSFM 15%**
Individual weight (g)	0.1195 ± .0012[a]	0.1453 ± 0.0047[b]	0.1503 ± 0.0037[b]	0.1404 ± 0.005[b]
Biomassa (g)	3.07 ± 0.01[a]	3.41 ± 0.29[ab]	4.05 ± 0.15[b]	3.77 ± 0.23[b]
Substrate reduction (%)	42.73 ± 1.59[a]	52.88 ± 3.88[ab]	50.83 ± 0.71[ab]	55.18 ± 8.49[b]
Bioconversion ratio (%)	5.67 ± 0.01[a]	6.29 ± 0.55[ab]	7.47 ± 0.27[b]	6.95 ± 0.42[b]
Growth rate (g/day)	0.0119 ± .0001[a]	0.0145 ± 0.0005[b]	0.0150 ± 0.0004[b]	0.0141 ± 0.0005[b]
Survival (%)	95.56 ± 2.94[a]	100.00 ± 0.00[a]	95.55 ± 2.22[a]	94.44 ± 5.56[a]

Noted: fPKM: 100% fermented Palm Kernel Meal; fPKM: fermented various ratios of Palm Kernel Meal (PKM) with Black Soldier Fly Meal (BSFM). Mean ± SE followed by different superscripts (a, b) in the same row indicate significantly different between groups (*P<0.05*)

Table 4. Proximate average value of BSFL fed various combinations of fermented Palm Kernel Meat (fPKM) and fermented Black Soldier Fly Meal (fBSFM)

Parameters (%)	Groups			
	fPKM	**fBSFM 5%**	**fBSFM 10%**	**fBSFM 15%**
Crude protein	33.98 ± 0.002[a]	34.72 ± 0.007[a]	35.38 ± 0.005[a]	36.11 ± 0.002[a]
Crude fat	25.02 ± 2.05[a]	26.09 ± 0.22[a]	18.25 ± 0.66[a]	25.34 ± 3.74[a]
Moisture	7.40 ± 1.12[a]	5.35 ± 0.19[a]	7.01 ± 0.07[a]	6.44 ± 0.93[a]
Ash	6.75 ± 0.07[a]	7.42 ± 0.02[a]	7.93 ± 0.08[a]	7.09 ± 0.01[a]
Crude fibre	19.48 ± 0.86[a]	22.58 ± 1.02[a]	18.55 ± 1.24[a]	22.46 ± 2.44[a]

Note: C: Control (fermented 100% palm kernel meal); P1-P3: Treatment substrate groups, various ratio (g) fermented of PKM: BSF. P1: 950:50 (5%); P2: 925:75 (7.5%); P3: 900:100 (10%). Mean ± SE followed by different superscripts (a, b) in the same row indicates significant differences between groups (*P<0.05*)

Discussion

The utilization of Black Soldier Fly Larvae (BSFL) in organic waste valorization has garnered significant attention due to their ability to convert low-quality substrates into high-quality dietary protein (Kuznetsova et al. 2022). The BSFL can effectively consume various organic wastes, such as fermented Palm Kernel Meal (fPKM) (Nugroho et al. 2024). Previous research has also shown that black soldier fly larvae can be successfully reared on a diet consisting of palm kernel meal (Nugroho et al. 2023). Black soldier fly larvae can convert organic waste into valuable protein and fat, making them highly beneficial for the feed industry. The development of black soldier fly larvae with palm kernel meal can enhance the circular economy by repurposing agricultural waste and mitigating the environmental effects of trash disposal (Ganda et al. 2019).

This study, the first of its kind, explores the use of fermented Black Soldier Fly Carcass Meal (fBSFM) in combination with fPKM as a substrate for BSFL growth. The findings suggest that the fermentation process of the fPKM and fBSFM could lead to significant improvements in several nutritional parameters. The increase in crude protein with higher BSFM content indicates the potential of BSFM as a valuable protein source. The stable fat content suggests that the fermentation process does not significantly alter the lipid profile, which could be a promising finding. The reduction in moisture content with higher BSFM proportions could benefit storage and shelf-life. The slight decrease in ash content might indicate a change in mineral composition. Furthermore, the increase in crude fiber with higher BSFM content suggests that BSFM contributes to the fiber content of the substrate, which could have positive implications for the nutritional value of the substrate.

Fermentation, a crucial biological process, positively affects the proximate composition of various substrates used for cultivating BSFL. These larvae have emerged as a promising solution for efficiently converting organic waste into valuable biomass, with potential applications in animal feed (da-Silva et al. 2024) and biofuel production (Mohan et al. 2023). The proximate composition of the substrate, which includes moisture, protein, fat, fiber, and ash content, is a crucial factor in determining the nutritional value and suitability of the substrate for BSFL. Researchers have explored the use of diverse organic substrates, such as brewery wet grains, cattle dung, and rumen content, as viable options for BSFL production (Herlina et al. 2021).

Based on the growth data of BSFL maintained at the fPKM and various levels of fBSFM, it is known that individual weight and growth rate of BSFL showed a significant difference between BSFL maintained without fBSFM and with any levels of fBSFM. These findings are significant in the fields of entomology, agriculture, and waste management. The BSFL has a higher individual weight and growth rate in the substrate with the addition of fBSFM than without fBSFM. The addition of BSFM might increase the protein content of the substrate when compared to the control group without the addition of BSFM. The control group, which only had fPKM as a substrate for BSFL, had a low protein content of about 6.33%, resulting in a low larval growth rate (Table 2). The current finding is supported by Tschirner and Simon (2015), who revealed that BSFL grown on dry grain waste substrate with a protein content of 31.2% produced a greater individual weight of larvae (270.7 mg wet weight) compared to BSFL reared on dry beet waste substrate (8.5%), which only produced an individual weight of 34.8 mg. The protein content of the substrate also has a linear effect on larval weight, i.e., the higher the protein content of the black soldier fly larval substrate, the greater the larval weight produced (Anasya et al. 2022; Kieβling et al. 2023). Further, Joly (2018) also reported that the substrate in the form of a mixture of kitchen waste enhanced the growth of BSFL. Mixing different types of organic waste can increase heterogeneity and improve substrate quality due to improved nutrients, substrate structure, and substrate composition (Spranghers et al. 2017; Joly 2018).

Furthermore, the BSFL grown with fPKM and various levels substituting the fBSFM showed significantly better growth parameters. This indicates that substituting fPKM with fBSFM improved the individual weight, biomass, substrate reduction, bioconversion rate, and growth rate. The level substitution of fPKM with fBSFM at a level higher than 5% for BSFL growth enhances substrate nutrients, especially protein and fat, improving the rate of bioconversion and biomass in the BSFL. A similar study by Nash and Chapman (2014) revealed that Mediterranean fruit fly larvae (*Ceratitis capitata* (Wiedemann, 1824)) grown on a low-protein but high-carbohydrate substrate experienced significantly slower larval development. Based on the study by Singh et al. (2021) also mentioned that BSFL grown on substrate in the form of a mixture of organic waste had a higher substrate/waste reduction rate

(72%) compared to vegetable waste substrate without a mixture (61%).

Substituting fPKM using fBSFM in the form of BSFM may increase nutrients and energy content. The energy content of the BSFL's substrate affected the efficiency of the substrate reduction ability of the BSFL (Nguyen et al. (2015). In the fPKM substrate (Table 2), the crude protein content is relatively low, around 6.33%, due to the lack of available nutrients. This finding is supported by Gold et al. (2020), who mentioned that BSFL reared on cow dung waste substrate with a protein content of 11.1% had a lower substrate reduction percentage (12.7%) when compared to poultry feed substrate with a protein content of 19.1% and a substrate reduction value of 67.6%. Similarly, Naser El Deen et al. (2023) reported that BSFL reared on pig manure substrate with a mixture of grass silage (crude protein content of 8.22%) had a low substrate reduction index (1.08 g/day). This occurs due to the low availability of nutrients, especially protein, in livestock waste, which plays a significant role in the production of metabolic waste in livestock.

The survival rate of BSFL in all groups was not significantly different. The high survival rate is one parameter used to determine the feasibility of an organic material as a substrate for BSFL. Broeckx et al. (2021) stated that on a laboratory scale, the survivability and growth of BSFL can be influenced by the conditions during breeding, such as light, temperature, humidity, larval density, harvest time, substrate composition, and the physico-chemical content of the substrate used. Previous research has also indicated that BSFL reared on high-protein (22%) and high-fat (9.5%) substrates exhibited accelerated development and increased survivability (Oonincx et al. 2015).

This study showed that the proximate content of crude protein, crude fat, moisture content, ash content, and crude fiber in BSFL fed with the substitution of fBSFM to fPKM (5, 7.5, and 10%) substrate did not provide significant differences. This indicates that the addition of BSFM to the fPKM substrate did not affect the nutrient content of the BSFL and only affected the BSFL growth rate. This result is similar to the study by Eggink et al. (2022), who reported that BSFL reared on shrimp waste substrate (*Pandalus borealis* Krøyer, 1838) contains protein (36.2%) and fat (22.9%) levels did not show any significant difference with proximate of the BSFL. In addition, Nguyen et al. (2015), similarly reported that BSFL reared on vegetable, fruit (mixed), and fish (mixed) substrates had lower protein and fat contents, namely on vegetable and fruit substrates (12.9%; 2.22%) and on fish substrates (19.4%; 11.6%).

In conclusion, adding 5% BSFM has been shown to enhance both the growth rate and individual weight of the larvae. A concentration of 7.5% MSFM can further boost biomass and bioconversion rates. Furthermore, a 10% concentration enhances the substrate reduction ability, thereby making the processing of black soldier fly flour waste more efficient. The addition of BSFM can also support the life of black soldier fly larvae, as evidenced by a low mortality rate of larvae and no significant differences observed between treatments. The addition of BSFM with a concentration of 5-10% did not result in a significant difference in the mean values of nutritional components of BSFL, which were approximately 34.72-35.38% crude protein, and 18.55-22.58.7% crude fiber, but significant differences for 18.25-26.09% crude fat, 5.35-7.01% moisture, and 7.42-7.93% ash. Further, the protein content of 35.38% is potentially suitable for livestock feed, suggesting that utilizing adult BSF waste as a substrate mixture for BSFL is worth considering.

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