nutritive value

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ORIGINAL RESEARCH ARTICLE

Nutritive value, material reduction, biomass conversion rate, and survival of black solider fly larvae reared on palm kernel meal supplemented with fish pellets and fructose

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Abstract

Black Soldier Fly Larvae (BSFL) is a potential for future feed source that can be grown in Palm Kernel Meal (PKM). To fortify the substrate of BSFL, fish feed and fructose can be added to induce BSFL metabolize polyunsaturated fatty acids which makes them more valuable. Their nutritive value and its growth performance reared in PKM supplemented with Fish Feed Pellet (FFP) and fructose has never been investigated. Present study aimed to evaluate the proximate, mineral, and fatty acid concentration, material reduction (MR), biomass conversion rate (BCR), and survival of BSFL grown on PKM, FFP, PKM:FFP (1:1), and fructose at various concentration (2.5, 5, 7.5, and 10%) in PKM:FFP. After 12 days of rearing, a high protein was observed in BSFL fed FFP (55.68 ± 2.30%), while moisture (2.74 ± 0.05%) and crude fat (7.69 ± 0.04%) were found on the BSFL grown in PKM:FFP. The fructose (2.5–7.5%) in PKM:FFP increased carbohydrate content of BSFL. The highest relative concentration of fatty acids was found in the BSFL fed PKM:FFP with 10% fructose. The addition of fructose in either PKM, FFP or its combination significantly affected ($P < 0.05$) on the fatty acid composition and some mineral accumulation, such as phosphorus (1.16 ± 0.04%), sodium (1.17 ± 0.01%), and iron (77.00 ± 1.00%) of the BSFL. However, the BSFL length, MR, and BCR were not significantly ($P > 0.05$) affected by the addition of fructose. The survival did not differ significantly ($P > 0.05$) among the BSFL grown in the various substrates. It is suggested that fructose is added to substrat of the BSFL to optimize fatty acid composition, mineral accumulation, proximate analysis without affected the growth and the survival of the BSFL.

Keywords Proximate analysis · Black soldier fly larvae · Palm kernel meal

Introduction

Among the numerous insects that may be farmed, black soldier fly larvae (BSFL), also known as *Hermetia illucens* L. (Diptera: Stratiomyidae), provide several nutritional benefits for livestock, fish culture, and sustainable agriculture (Ee et al. 2022; Ouko et al. 2022; Seyedalmoosavi et al. 2022). The BSF is found globally in various climates and nutrient-rich environments with an abundance of decaying animal and plant remnants (Khairuddin et al. 2022; Panikkar et al. 2022; Rehman et al. 2022). BSFL have several beneficial properties, including a relatively high protein and fat content, the ability to grow on a variety of waste materials that are unfit for use as human food (da Silva and Hesselberg 2020). BSFL nutritional content can reach up to 40% protein (Ndotono et al. 2022) and 30% lipid (Lin et al. 2022) and varies depending on the substrate utilized

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for growth (Newton et al. 2005; St-Hilaire et al. 2007). Furthermore, the substrate type influences the production and subsequent nutritional value of BSFL (Fischer and Romano 2021; Lalander et al. 2019; Truzzi et al. 2020). BSFL typically grow faster on a diet with a balanced ratio of digestible protein to carbohydrate (Cammack and Tomberlin 2017), although diets with a greater protein content may boost larval growth and/or result in bigger larvae (Lalander et al. 2019; Nguyen et al. 2015; Tinder et al. 2017).

During replanting in oil palm farms, biomass consisting of palm fronds and trunks is produced. Empty fruit bunches, palm kernel meal (PKM), and palm oil mill effluent are all produced in palm oil mills during the production of fresh fruit bunches (FFB) into crude palm oil. With oil palm farms, FFB production facilities, and palm oil mills spread over 22 provinces in Indonesia, these waste products are abundant. These waste products are commercially valuable since they can be turned into alternative fuels, fertilizers, chemical compounds, and biomaterials, and innovative research is needed to boost the value of PKM and make the oil palm industry more sustainable (Hambali and Rivai 2017). Azizi et al. (2021) revealed that palm kernel cake contains about 14–18% of crude protein, 12–20% crude fiber, 3–9% ether extract, and several types of minerals. In addition, Balandrán-Quintana et al. (2019) stated that PKM has a protein content ranging from 14.4 to 20%, with high amounts of both carbohydrates (50.3%) and crude fiber (16.7%). In addition, though the palm kernel waste is high in cellulose, it is interesting to note that there are signs that BSFL have some capacity to digest fibers such as cellulose, hemicellulose, and lignin, that this ability may even improve BSFL production when combined in the appropriate manner (Beesigamukama et al. 2020, 2021; Rehman et al. 2017; Romano 2022).

Moreover, the presence of a specific carbohydrate such as wheat starch in the substrate used to grow BSFL was found to induce the lipid content of BSFL. According to Hoc et al. (2020), diets high in starch have been shown to enhance the production of certain saturated fatty acids, such as capric acid (C10), lauric acid (C12), and myristic acid (C14), which may lead to an increase in the lipid content of BSFL. Indeed, the consumption of bread or dough resulted in a significant increase of C12 in BSFL when compared to the consumption of fish or spent coffee grounds (Fischer et al. 2022; Spranghers et al. 2017), both of which contain a lower amount of digestible carbohydrates.

Further, adding mono- and disaccharides as well as starch had no influence on growth performance. Larvae crude lipid levels were considerably raised by wheat starch and lowered by either galactose or xylan addition, although fatty acid profiles were mostly unchanged, with lauric acid dominating. Furthermore, adding galactose and xylan in the substrate decreased the lipid profile of BSFL and their bioconversion efficiency (Cohn et al. 2022). Nevertheless, BSFL lack polyunsaturated fatty acids such as eicosapentaenoic acid or docosahexaenoic acid, which makes them less valuable. To resolve this issue, chicken feed was used as a BSFL substrate and fortified with squid liver oil (SLO) (Tirtawijaya and Choi 2022).

Previous studies stated that BSFL have several advantages especially in improving the growth of chickens (Khaemba et al. 2022), beef cattle (Drewery et al. 2022), and fish culture (Mohan et al. 2022) fed with BSFL protein meal. Besides protein, the fat content of BSFL meets the requirements for fish culture established by the United Nations Food and Agriculture Organization (FAO) (Pérez-Pacheco et al. 2022). Lu et al. (2022) stated that BSFL can replace conventional protein feed such as soybean meal and fish meal. The oil from BSFL may also replace the fish oil in siberian sturgeon fish (*Acipenser baerii*) and improve sturgeon growth indices (Rawski et al. 2020). Thus, BSFL can be developed as a promising alternative feed ingredient for fish, specifically as a substitute for fish meal and oil (Nugroho and Nur 2018).

Although some studies have evaluated the growth of BSFL in several substrates, the growth and nutritional value of BSFL reared in PKM and FFP supplemented with various concentrations of fructose have not been assessed. Thus, the present study aimed to determine the growth and proximate content of BSFL fed PKM and FFP supplemented with 2.5, 5, 7.5, or 10% fructose.

Materials and methods

Stock BSFL colony

A population of BSFL (7-day old larvae) having an average weight of 0.05 ± 0.004 g and average length of 12.43 ± 0.25 mm was obtained from a local BSF farmer in Samarinda City, East Kalimantan, Indonesia. The BSFL were derived from second-generation flies reared with fermented PKM. The BSFL were kept in a plastic box equipped with a screen mesh lid (1.5-mm mesh) that provided adequate access to the sunlight spectrum and maintained a temperature of 28 °C. All the plastic box was placed in the room that equipped with air con that set to 28 °C.

Preparation of substrate and larvae feeding

In total of seven treatments that consisted of only palm kernel meal, only fish feed pellets or a combination of PKM:FFP and added with various levels of fructose is described in the Table 1:

Palm Kernel Meal was chosen as the substrates based on its availability in Samarinda City, East Kalimantan, Indonesia, with the possibility of its future usage in large-scale industrial

Table 1 Composition of seven treatments used for black soldier fly larva rearing

Treatments	Palm Kernel meal (PKM)	Fish Feed Pellet (FFP)	Fructose addition (%)
1	+	-	-
2	-	+	-
3	+	+	-
4	+	+	2.5
5	+	+	5
6	+	+	7.5
7	+	+	10

Palm Kernel Meal and Fish Feed Pellet was combined at a ratio 1:1

BSFL production in Samarinda, Indonesia. Meanwhile the addition of FFP is to improve the nutritional value of the PKM, to support the growth of the BSFL. To obtain sufficient supporting data, proximate and mineral analysis was also performed for each type of substrate. Tables 2 and 3 present the results of the substrate proximate and mineral analysis.

Five hundred grams of BSFL (7-day-old larvae) was added to each kilogram of non-fermented substrate in the beginning of the study. Three replicates were prepared for each substrate and randomly placed in a 23 × 15 cm plastic box in a room (temperature 28 ± 2 °C, relative humidity 65 ± 5%) throughout the growth period. The various substrates that was prepared for all groups were given during the study (12 days), following previous work by Guo et al. (2021). Around 250 mL distilled water was sprayed per kilogram of substrate to maintain a moisture level of 65–70%. After the BSFL reached the prepupal stage, they were collected. The BSFL prepupae were oven-dried at 60 °C for 48 h and crushed. The BSFL meal and its oil were separated using an oil extractor (RG-307, Jiangsu, China). The defatted BSFL meal was then kept in a refrigerator (Sharp Chest Freezer Series FRV-300, Sharp, Indonesia) at a temperature of –20 °C for further proximate and mineral analysis, while the BSFL oil was used for fatty acid composition analysis.

Determination of larval growth, bioconversion, and survival

To evaluate the larval growth, material reduction (MR), biomass conversion rate (BCR), and survival (%) of the BSFL grown in the different substrate treatments, a separate experiment with the following protocol was performed. A total of 560 BSFL (initial weight 0.05 ± 0.004 g and length 12.43 ± 0.25 mm) were randomly divided into seven groups (four replications; n = 20 BSFL per replication) of substrates comprising PKM, FFP, PKM:FFP (1:1), PKM:FFP (1:1) PKM with FFP in a 1:1 ratio supplemented with various concentrations of fructose (2.5, 5, 7.5, and 10%). Each group of BSFL was placed in a small plastic bottle containing 60 g of substrate. Thirty mL of distilled water were added every day to maintain the humidity. The BSFL were reared for 12 days at room temperature (28 ± 2 °C) and a relative humidity of 65 ± 5%. At the end of Day 12, all larvae were counted for survival, and their final weights (g) and lengths (mm) were measured using a standard digital weight balance and digital calipers. Meanwhile, the remaining substrate was removed with a brush and assessed for wet weight and dry weight (dry matter basis). The substrate was dried using an industrial oven at 60 °C for 24 h. The dry substrate was used to calculate MR and BCR. Meanwhile, total dry matter of larvae was calculated by oven-dried the BSFL at 60 °C for 48 h and crushed.

The percentage of MR (dry matter basis) and the BCR were determined using the equation used previously by Romano et al. (2022):

$$\text{Percentage of MR (dry matter basis)} = \left(1 - \frac{\text{Initial substrate}}{\text{Final substrate}}\right) \times 100$$

The BCR was calculated based on the percentage of substrate conversion into biomass using the following equation:

$$\text{BCR} = \left(\frac{\text{Total dry matter of larvae}}{\text{Initialsubstrate} - \text{remaining substrate}}\right) \times 100$$

Table 2 Initial proximate analysis (%, as-is basis) of various substrates used for black soldier fly larva rearing (n = 3)

	PKM	FFP	PKM:FFP	PKM:FFP(F2.5)	PKM:FFP(F5)	PKM:FFP(F7.5)	PKM:FFP(F10)
Crude Protein	15.19	16.32	16.38	16.67	16.11	15.58	15.98
Crude Fat	13.28	10.70	10.24	10.92	10.72	10.43	10.19
Carbohydrate	60.11	61.89	62.57	61.22	62.10	63.08	63.11
Ash	5.68	5.53	5.43	5.58	5.49	5.42	5.36
Moisture	5.73	5.54	5.36	5.59	5.56	5.48	5.35

PKM Palm Kernel Meal, *FFP* Fish Feed Pellets, PKM:FFP in a 1:1 ratio supplemented with 2.5, 5, 7.5, and 10% fructose. Data shows as Mean

Table 3 Mineral content analysis (%, as-is basis) of initial substrates used for black soldier fly larva rearing (n = 3)

	PKM	FFP	PKM:FFP	PKM:FFP(F2.5)	PKM:FFP(F5)	PKM:FFP(F7.5)	PKM:FFP(F10)
Phosphorous (P)	0.30	0.4	0.42	0.39	0.54	0.48	0.41
Calcium Ca)	0.49	0.69	0.56	0.57	0.60	0.60	0.63
Potassium (K)	0.53	0.78	0.74	0.66	0.73	0.74	0.67
Magnesium (Mg)	0.04	0.04	0.04	0.04	0.03	0.04	0.03
Sodium (Na)	0.16	0.08	0.07	0.13	0.11	0.13	0.13
Iron (Fe)	103.50	18.00	51.00	58.00	51.50	57.00	53.50

PKM Palm Kernel Meal, *FFP* Fish Feed Pellets, PKM:FFP in a 1:1 ratio supplemented with 2.5, 5, 7.5, and 10% fructose. Data shows as Mean

The survival rate of the BSFL was determined during the study using the equation

$$\text{Survival}(\%) = \frac{\text{Number of surviving BSFL}}{\text{Number of initial BSFL}} \times 100$$

Mineral content analysis

The content of minerals such as phosphorus, calcium, potassium, magnesium, sodium, and iron in the defatted BSFL meal from all groups was measured. Each sample was ashed at 500 °C for 5 h, until a consistent weight was attained (Muffle furnace, BF-02/15, SM Indo, Banten, Indonesia) and then allowed to cool to room temperature. Thereafter, 5 mL of 1 M HNO_3 was added. The solution was then filtered into a 100-mL volumetric flask that had been filled to capacity with 1 M HNO_3. Mineral content was measured using an atomic absorption spectrophotometer (AA6300, Shimazdu, Japan).

Fatty acid composition analysis

The BSFL oil was obtained from the separation of BSFL dried using an oil extractor (RG-307, Jiangsu, China). The BSFL prepupal were oven-dried at 60 °C for 48 h and crushed. The BSFL oil was separated from the BSFL meal using an oil extractor (RG-307, Jiangsu, China). The resulting oil was tested for fatty acid composition using gas chromatography-mass spectrometry (GC/MS; Agilent Technologies, CA, USA). Before analysis, fatty acids in oil samples were converted to fatty acid methyl esters (FAMEs) using the Ichihara and Fukubayashi (2010) technique. In brief, 0.20 mL of toluene, 1.50 mL of methanol, and 0.30 mL of 8.0% (w/v) concentrated hydrochloric acid were combined with oil samples (0.015 g). For 5 min, the mixture was incubated at 100 °C. The mixture was then combined with 1 mL of hexane and 1 mL of water. For analysis, the hexane layer (top layer) was separated. Further, a HP-5MS capillary column (30 m 0.25 mm, 0.25 m) was employed, and helium was used as the carrier gas at 1 mL/min. The impact energy of electrons was 70 eV. The injection volume was 1.0 L with a split ratio of 15:1 and an input temperature of 280 °C. The column temperature was set to 165 °C, followed by a 4 °C per minute ramp to 290 °C oven temperature. The temperature of the transfer line was 290 °C, and the temperatures of the ion sources were MS Quad 150 °C and MS source 230 °C, respectively. The mass range scanned was 29–550 m/z, with the detector voltage set at 1150 V. The observed components were identified by comparing retention durations to their standards and matching their mass spectra with the reference spectra in the NIST 98 Mass Spectral Library.

Proximate analysis

The defatted BSFL meal from each group was prepared for proximate analysis, including analysis of crude protein, crude fat, ash, and moisture content, utilizing the established technique of the Association of Official Analytical Chemists (AOAC), AOAC International, 17th ed v.2, Gaithersburg, Md (Horwitz 2000). Carbohydrate content was calculated by difference using the Pearson's Chemical Analysis of Foods technique (Ayoola and Adeyeye 2010).

Statistical analysis

Data obtained were evaluated using SPSS ver. 22 (SPSS Inc., USA). The Shapiro–Wilk was used to detect the normality of the obtained data. A one-way analysis of variance (ANOVA) was used. Any significant difference was found in the ANOVA, Duncan's multiple range test (DMRT), was performed to determine the significant differences among the means, with significancy P-values at 0.05.

Results

Larval growth, bioconversion, and survival

BSFL reared on PKM:FFP (10% fructose) had the highest weight (0.24 ± 0.004 g; Fig. 1A), while the length of the

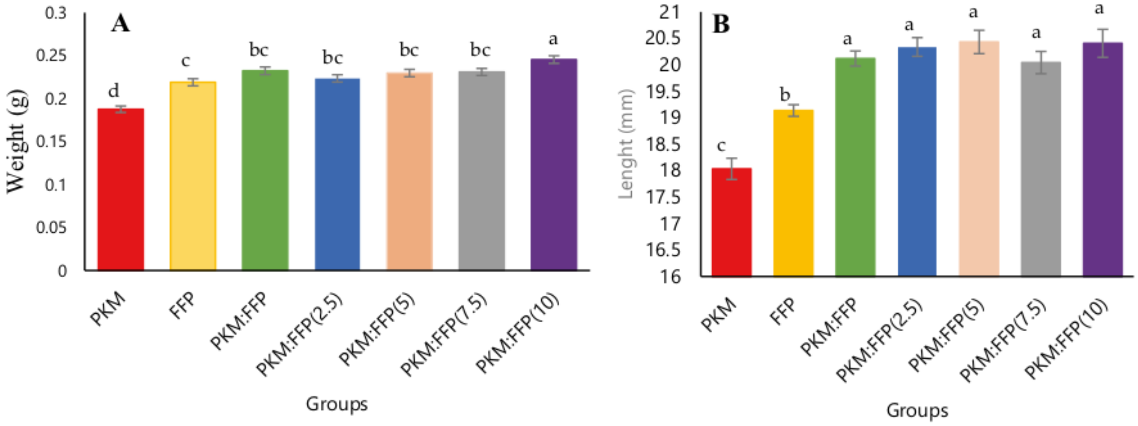

Fig. 1 Mean ± SE of **A** weight and **B** length of black soldier fly (*Hermetia illucens*) larvae after 12 days of being fed palm kernel meal (PKM) and fish feed pellets (FFP) supplemented with various concentration of fructose (2.5, 5, 7.5, and 10%). Different letters above each bar indicate significant differences ($P < 0.05$)

BSFL was not affected by the addition of any concentration of fructose (Fig. 1B). Both the percentage of MR and BCR were not affected by the addition of any concentration of fructose but showed significant differences to those of BSFL reared in PKM. The MR by BSFL was from 68.58 ± 1.06% in the PKM:FFP group to 88.60 ± 1.23% in the PKM:FFP (10% fructose) group. Furthermore, the survival of BSFL ranged from 93.00 ± 0.70 to 97.00 ± 2.38%. However, there was no statistically significant difference between the substrate groups in this regard (Table 4).

Mineral content

The BSFL reared in PKM:FFP has the highest phosphorus, while BSFL grown in FFP showed the highest accumulation of sodium in their body. The substrate type had no significant effect on the calcium, potassium, and magnesium levels. The addition of more than 5% fructose in the PKM:FFP substrate resulted in significantly higher iron in the BSFL than in other groups (Table 5).

Fatty acid composition

In total, nine fatty acids were detected in the BSFL oil (Table 6). The addition of more than 2.5% fructose to the PKM:FFP reduced lauric and linolelaidic acid content but increased arachidic acid and nervonic acid content in the BSFL. The myristoleic acid content of the BSFL slightly improved when they were grown in PKM:FFP with or without fructose addition at any concentration. The BSFL reared in PKM only had the highest palmitoleic, linolelaidic, and α-linolenic acid content. Significantly higher eruchic acid and docosadienoic acid content were found in BSFL grown in the PKM:FFP substrate with any concentration of fructose. On the other hand, docosadienoic acid was not detected in BSFL fed PKM.

Furthermore, adding 2.5–10% fructose in PKM:FFP greatly enhanced the relative concentration of unsaturated fatty acids. The highest relative concentration of unsaturated fatty acids was found in the BSFL reared in PKM:FFP with 10% fructose (Fig. 2).

Table 4 Material reduction (MR), biomass conversion rate (BCR), and final survival of black soldier fly (*Hermetia illucens*) larvae after 12 days of feeding on palm kernel meal and fish feed pellets in combination with various concentration of fructose (n = 3)

	PKM	FFP	PKM:FFP	PKM:FFP(F2.5)	PKM:FFP(F5)	PKM:FFP(F7.5)	PKM:FFP(F10)
Material reduction (MR) (%)	76.85 ± 6.93^{b}	71.03 ± 3.60^{b}	68.58 ± 1.06^{b}	86.62 ± 0.69^{a}	87.27 ± 1.02^{a}	87.24 ± 0.86^{a}	88.60 ± 1.23^{a}
Biomass conversion rate (BCR)	32.55 ± 3.05^{b}	40.77 ± 1.93^{a}	43.33 ± 1.01^{a}	32.42 ± 0.49^{b}	33.33 ± 0.58^{b}	32.21 ± 0.79^{b}	32.21 ± 0.94^{b}
Survival (%)	94.00 ± 2.94^{a}	95.25 ± 0.47^{a}	93.00 ± 0.70^{a}	95.00 ± 2.88^{a}	96.50 ± 1.25^{a}	97.00 ± 2.38^{a}	96.50 ± 1.25^{a}

PKM Palm Kernel Meal, *FFP* Fish Feed Pellets, PKM:FFP in a 1:1 ratio supplemented with 2.5, 5, 7.5, and 10% fructose. Different superscripted letters in each row indicate significant differences ($P < 0.05$). Material reduction (MR), biomass conversion rate (BCR)

Table 5 Mineral content (%, as-is basis) of defatted black soldier fly larva meal reared in palm kernel meal and fish feed pellets with/without fructose addition for 12 days (n = 3)

	PKM	FFP	PKM:FFP	PKM:FFP(F2.5)	PKM:FFP(F5)	PKM:FFP(F7.5)	PKM:FFP(F10)
Phosphorus (P)	0.93 ± 0.05^{d}	0.97 ± 0.01^{cd}	1.16 ± 0.04^{a}	0.98 ± 0.03^{cd}	1.05 ± 0.04^{b}	1.12 ± 0.01^{ab}	1.07 ± 0.01^{bcd}
Calcium Ca)	2.28 ± 0.01^{a}	2.23 ± 0.01^{a}	2.23 ± 0.01^{a}	2.20 ± 0.09^{a}	2.10 ± 0.10^{a}	2.28 ± 0.05^{a}	2.24 ± 0.01^{a}
Potassium (K)	1.01 ± 0.05^{a}	1.04 ± 0.01^{a}	1.03 ± 0.01^{a}	1.07 ± 0.02^{a}	1.05 ± 0.01^{a}	1.04 ± 0.01^{a}	1.19 ± 0.19^{a}
Magnesium (Mg)	0.05 ± 0.01^{a}	0.05 ± 0.00^{a}	0.06 ± 0.01^{a}	0.05 ± 0.00^{a}	0.06 ± 0.01^{a}	0.04 ± 0.00^{a}	0.05 ± 0.01^{a}
Sodium (Na)	0.14 ± 0.01^{c}	1.17 ± 0.01^{a}	1.13 ± 0.01^{b}	0.07 ± 0.01^{d}	0.06 ± 0.01^{e}	0.06 ± 0.01^{e}	0.05 ± 0.01^{e}
Iron (Fe)	49.50 ± 0.50^{c}	25.80 ± 0.21^{d}	72.50 ± 0.50^{b}	72.50 ± 1.50^{b}	70.00 ± 1.00^{b}	77.00 ± 1.00^{a}	79.50 ± 0.50^{a}

Mean ± standard error followed by different superscripts in the same row indicate significant differences between groups ($P < 0.05$). *PKM* Palm Kernel Meal, *FFP* Fish Feed Pellets, PKM:FFP in a 1:1 ratio supplemented with 2.5, 5, 7.5, and 10% fructose

Proximate analysis

The crude protein content of BSFL reared in FFP was significantly higher ($P < 0.05$) than that of other groups, while BSFL reared in PKM:FFP shown high crude fat content ($P < 0.05$) in comparison to other groups. Though the addition of fructose increased carbohydrate content, the highest added concentration of fructose (10%) in the PKM:FFP reduced the BSFL carbohydrate content. Furthermore, adding more than 5% fructose in the substrate (PKM:FFP) significantly increased ($P < 0.05$) the ash content of the BSFL, while BSFL reared in PKM:FFP had the highest moisture content (Table 7).

Discussion

Insect farming is gaining momentum in tropical areas, with the black soldier fly (BSFL) offering advantages for animal nutrition. BSFL have a high protein and fat content, can grow on waste items, and are not regarded as a pest or nuisance species. This work evaluated the nutritional aspects, growth, MR, and BCR of BSFL reared in PKM, FFP, and their combination with/without fructose addition.

Larval growth, bioconversion, and survival

The present study revealed that BSFL fed PKM, FFP, and a combination thereof with or without fructose addition showed an increase in weight but not length. The addition of 10% fructose greatly affected BSFL weight. A past study also mentioned that fruit waste, which may contain high glucose and fructose levels, had an advantageous effect on the growth of BSFL (Pérez-Pacheco et al. 2022). Furthermore, the type of substrate is critical for optimizing BSFL bioconversion activity (Fadhillah and Bagastyo 2020; Tanga et al. 2021; Wang et al. 2020). BSFL, like other living species, need nutrients for growth. As a result, to improve bioconversion efficiency, the BSFL must feed on organic wastes that has a high in digestible nutritional elements such as palm waste (Kinasih et al. 2020; Latifah et al. 2021; Liew et al. 2022). This bioconversion of the palm kernel meal by BSFL is reflected as material reduction and the improvement of the BSFL growth indices.

Table 6 Fatty acid composition of black soldier fly larva reared in palm kernel meal and fish feed pellets with/without fructose addition for 12 days (n = 3)

	PKM	FFP	PKM:FFP	PKM:FFP(F2.5)	PKM:FFP(F5)	PKM:FFP(F7.5)	PKM:FFP(F10)
Lauric acid (C12:0)	19.75 ± 0.82^{a}	19.04 ± 0.80^{a}	19.04 ± 0.80^{a}	14.43 ± 0.85^{b}	11.77 ± 0.13^{c}	11.29 ± 0.21^{c}	11.22 ± 0.02^{c}
Myristoleic acid (C14:1)	3.46 ± 0.25^{b}	3.90 ± 0.29^{a}	4.90 ± 0.29^{a}	4.67 ± 0.12^{a}	4.43 ± 0.08^{a}	4.35 ± 0.36^{a}	4.53 ± 0.14^{a}
Palmitoleic acid (C16:1)	21.11 ± 0.56^{a}	18.17 ± 0.57^{b}	18.22 ± 0.48^{b}	11.16 ± 0.50^{d}	10.89 ± 0.27^{d}	10.76 ± 0.20^{d}	14.71 ± 0.25^{c}
Linolelaidic acid (C18:2n6t)	26.08 ± 0.20^{a}	23.02 ± 0.47^{b}	23.35 ± 0.26^{b}	20.24 ± 0.36^{c}	14.56 ± 0.31^{cd}	13.96 ± 0.08^{e}	15.13 ± 0.28^{d}
α-Linolenic acid (C18:3n3)	17.77 ± 1.67^{a}	14.80 ± 0.22^{b}	14.75 ± 0.22^{b}	10.69 ± 0.46^{c}	9.68 ± 0.18^{c}	8.51 ± 0.55^{c}	7.33 ± 0.11^{d}
Arachidic acid (C20:0)	3.28 ± 0.09^{b}	3.32 ± 0.18^{b}	3.13 ± 0.04^{b}	3.24 ± 0.17^{b}	6.67 ± 0.19^{a}	6.55 ± 0.31^{a}	6.36 ± 0.11^{a}
Eruchic acid (C22:ln9)	3.99 ± 0.16^{c}	8.65 ± 0.26^{b}	8.69 ± 0.88^{b}	22.43 ± 0.50^{a}	22.08 ± 0.30^{a}	22.19 ± 1.71^{a}	22.33 ± 0.28^{a}
Docosadienoic acid (C22:2)	0.00 ± 0.00^{d}	4.92 ± 0.19^{c}	4.82 ± 0.17^{c}	10.88 ± 0.41^{b}	11.27 ± 0.11^{a}	12.88 ± 0.28^{a}	12.29 ± 0.08^{a}
Nervonic acid (C24:ln9)	3.23 ± 0.11^{b}	2.65 ± 0.16^{b}	2.45 ± 0.15^{b}	2.19 ± 1.23^{b}	8.57 ± 0.26^{a}	8.48 ± 0.96^{a}	6.67 ± 0.27^{a}

Mean ± standard error followed by different superscripts in the same row indicate significant difference between groups ($P < 0.05$). *PKM* Palm Kernel Meal, *FFP* Fish Feed Pellets, PKM:FFP in a 1:1 ratio supplemented with 2.5, 5, 7.5, and 10% fructose

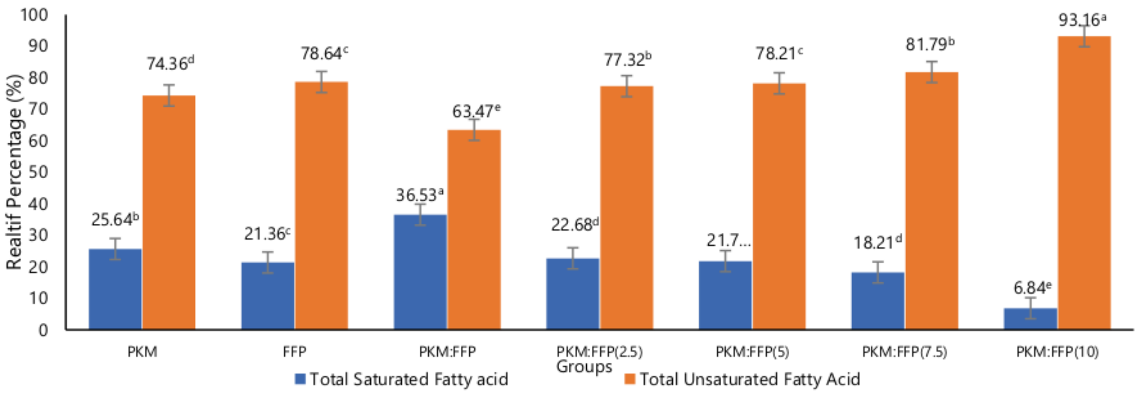

Fig. 2 Relative concentration of fatty acids in black soldier fly larvae reared in various substrates for 12 days. PKM = Palm Kernel Meal; FFP = Fish Feed Pellets; PKM:FFP in a 1:1 ratio supplemented with 2.5, 5, 7.5, and 10% fructose

A previous study showed that BSFL reared on fruit waste for 15 days had an average bioconversion rate of about 12.03%. In addition, it is important to be noted that the recycling performance of BSFL regarding material reduction and bioconversion may vary with different substrates and rearing conditions (Banks et al. 2014; Cai et al. 2019; Manurung et al. 2016), which also could affect their survival rate (Banks et al. 2014; Meijer et al. 2021; Taufek et al. 2021; Van der Fels-Klerx et al. 2016). Lastly, in term to optimize the growth performance of BSFL in the farming, the addition of fructose in the substrat of BSFL is recommended.

Mineral content

BSFL have the capability to accumulate significant amounts of minerals from various substrates. Minerals such as calcium, potassium, and magnesium in the defatted BSFL meal after 12 days of rearing in various substrates did not differ significantly in content. However, iron levels were significantly higher in the defatted BSFL meal grown in substrate with more than 5% fructose than in other groups. Based on the mineral composition used in this study, both PKM and FFP had large amounts of iron (Table 2). Previous studies stated that the mineral content of palm kernel cake is about 835–6130 ppm (Alimon 2004; Bárcena-Gama et al. 2022). This proofed that BSFL have the capability to deposit iron sources from the substrate (PKM and FFP). Similar past results revealed that BSFL grown in brewer's waste and standard diet or pig manure could take up a significant amount of minerals (Liu et al. 2018). In addition, BSFL treated with different substrates showed various mineral deposit patterns, showing a huge amount of turnover of minerals, especially potassium (Adebayo et al. 2021; Paul et al. 2023).

Meanwhile, BSFL grown on the various substrates showed significantly different accumulation of phosphorus. Present study revealed that the supplementation of fructose in the substrate of BSFL reduced the phosphorus accumulation. There is some evidence to suggest that a diet high in fructose may be linked to changes in phosphorus metabolism. Some research revealed that fructose consumption may alter the way that the body regulates phosphorus levels, leading to a decrease in serum phosphorus levels (Hallfrisch et al. 1986; Mayes 1993; Wong 2022). Phosphorus is an

Table 7 Proximate analysis of defatted black soldier fly larva meal reared in palm kernel meal and fish feed pellets with/without fructose addition for 12 days (n = 3)

	PKM	FFP	PKM:FFP	PKM:FFP(F2.5)	PKM:FFP(F5)	PKM:FFP(F7.5)	PKM:FFP(F10)
Crude protein (%)	48.82 ± 0.29^{b}	55.68 ± 2.30^{a}	51.19 ± 0.60^{b}	50.23 ± 1.33^{b}	50.02 ± 0.25^{b}	49.58 ± 1.01^{b}	51.88 ± 0.22^{b}
Crude fat (%)	5.31 ± 0.38^{b}	4.30 ± 0.16^{c}	7.69 ± 0.04^{a}	4.45 ± 0.09^{c}	4.50 ± 0.06^{c}	4.77 ± 0.24^{cb}	4.72 ± 0.01^{cb}
Carbohydrate (%)	32.04 ± 0.27^{a}	26.74 ± 2.41^{b}	26.80 ± 0.52^{b}	31.94 ± 1.43^{a}	32.32 ± 0.37^{a}	32.23 ± 1.46^{a}	29.58 ± 0.26^{ab}
Ash (%)	11.36 ± 0.04^{cd}	11.67 ± 0.15^{bc}	11.56 ± 0.11^{bcd}	11.44 ± 0.14^{bcd}	11.29 ± 0.13^{c}	11.77 ± 0.01^{c}	12.43 ± 0.02^{d}
Moisture (%)	2.45 ± 0.16^{a}	1.59 ± 0.13^{cb}	2.74 ± 0.05^{a}	1.92 ± 0.03^{b}	1.86 ± 0.01^{b}	1.64 ± 0.22^{cb}	1.38 ± 0.04^{c}

Mean ± standard error followed by different superscripts in the same row indicate significant differences between groups ($P < 0.05$). *PKM* Palm Kernel Meal, *FFP* Fish Feed Pellets, PKM:FFP in a 1:1 ratio supplemented with 2.5, 5, 7.5, and 10% fructose essential component in various physiological functions of animals. It plays a crucial role in maintaining osmotic and acidic-alkaline equilibria, constructing membranes, reducing muscle mass, facilitating neuro-signaling, supporting enzymatic activity, enabling metabolic reactions, and aiding in the construction of proteins. Its presence is vital for the proper functioning of these biological processes in animals (Shumo et al. 2019).

Fatty acid composition

The present study found a high relative concentration of some fatty acids in the BSFL reared in PKM, namely lauric acid (C12:0), palmitoleic acid (C16:1), and linolelaidic acid (C18:2n6t). According to Oonincx et al. (2015), the main fatty acid in BSFL was lauric acid (C12:0), which distinguishes BSFL from other insects such as crickets and mealworms. This is thought to be due to BSFL converting a high proportion of carbohydrates into lauric acid (Spranghers et al. 2017). However, this study revealed that the addition of fructose in the PKM:FFP substrate reduced lauric acid in the BSFL. Similar studies regarding the effect of fructose on lipid metabolism were performed by Abdel-Sayed et al. (2008); Prager and Ontko (1976), stating that fructose has a direct effect on fatty acid oxidation. Further, the high diet of fructose significantly reduces lipid oxidation and lipolysis. The high diet of fructose also raises lactate levels, and the subsequent rise in lactate utilization may help to reduce lipid oxidation. In addition, lipid oxidation has negative impacts on the animal health, hence the reduce of lipid oxidation may have a beneficial for the health status of the animal (Leong 2021). Thus, it is suggested that fructose should not be added to the combination of PKM:FFP if the BSFL is pointed to produce lauric acid.

In animals, lauric acid is promptly oxidized rather than retained in the liver, resulting in reduced feed intake in several fish (Belghit et al. 2019) and anti-obesity characteristics in mammals (St-Onge et al. 2008; St-Onge and Jones 2002). Wang et al. (2015) also revealed that medium-chain fatty acids may be beneficial for abdominal fat reduction due to their preferred usage in energy consumption over long-chain saturated or unsaturated fatty acids. Furthermore, medium-chain fatty acids, such as lauric acid, are known to have antibacterial effects on gut flora (Schiavone et al. 2017; Zeitz et al. 2015). Thus, insect oils high in lauric acid might have an effect on growth performance and intestinal health in animals. In addition, BSFL oil having a high content of lauric acid (C12:0) is similar to coconut oil (Li et al. 2016; Ushakova et al. 2016).

Furthermore, palmitoleic acid (C16:1), known as an ω-7 monounsaturated fatty acid, is a pivotal fatty acid used in pharmaceuticals. It is thought to have anti-thrombotic properties, which may aid in stroke prevention (Abraham et al. 1989). It is now derived mostly from macadamia oil (*Macadamia integrifolia*), which contains 17% palmitoleic acid. Palm Oil Research Institute of Malaysia (PORIM) previously discovered that *Elaeis guineensis* protoplasts can synthesize up to 30% palmitoleic acid in their total lipids (Parveez 2000; Sambanthamurthi et al. 1996). This demonstrates palms' natural propensity for synthesizing large amounts of this fatty acid (Norde et al. 2019; Parveez et al. 2012).

The current finding is also demonstrated that BSFL could convert the PKM and FFP substrate into palmitoleic acid in high amounts. A high palmitoleic acid content (more than 15% of total fatty acids) also characteristically separates Diptera from other orders of insects (Aguilar 2021; Barlow 1964). Palmitoleic acid is abundant in the larval lipids of five of the eight lepidopteran species (Bracken and Harris 1969; Thomas and Kiin-Kabari 2022). In addition, linolelaidic acid (C18:2n6t), an ω-6 trans fatty acid (TFA), was also found in relatively high concentrations in BSFL reared in PKM and FFP without fructose addition. A past study also found the ω-6 TFA at a concentration of 0.99 g/100 g in BSF prepupae reared in a mix of vegetable and fruit wastes (Giannetto et al. 2020). Further, there is no docosadienoic acid was detected in the BSFL grown in PKM. This results similar to past study that growing BSFL in dough (Ewald et al. 2020; Fischer et al. 2022, 2021), vegetables (Jucker et al. 2017), and cereals (Gao et al. 2019) had no effect on their docosadienoic acid content.

On the other hand, the addition of fructose any concentration between 2.5–10% showed significantly higher eruchic acid or known as a monounsaturated ω-9 fatty acid (MUFA/Mono-Unsaturated Fatty Acid). Similar finding has been revealed by Shi et al. (2023), stating that total MUFA were significantly enhanced by adding fructose in the free fatty acid pool in high fat diet of mice. Thus, industrial farming of BSFL, the addition of fructose in the substrate of BSFL may have a beneficial to produce MUFA, especially eruchic acid.

In general, the mechanism by which fructose affects fatty acid metabolism is relatively unknown. However, Giannetto et al. (2020) revealed that the different fatty acid profiles of BSFL at the prepupal stage may involve the modulation of the expression of lipid metabolism-related genes during larval development. In addition, glucose and lipid metabolism are connected by many other pathways (Parhofer 2015), while dietary fructose in the liver is involved in gene expression during lipogenesis in animal models (DiStefano 2020).

Proximate composition

The FFP substrate led to the production of high crude protein content (55.68 ± 2.30%) in the defatted BSFL meal. This crude protein content was similar to that of defatted BSFL meal grown in food waste (Ebeneezar et al. 2021) but significantly higher than that of BSFL reared in agroindustry by-products (39–48%) (Zulkifli et al. 2022) and rice straw (34.62%) (Pamintuan et al. 2020). In BSFL, it is preferred that BSFL has to be grown in organic waste to produce a high protein content. The high content of protein in the BSFL can be an alternative way to substitute fish meal which is more expensive, unsustainable, and scarce (Kishawy et al. 2022). Presently, in aquafeed industry, fish meal can be partly or fully replaced with other source of high protein, such as BSFL meal (Priyadarshana and Ruwandeepika 2021; Xiao et al. 2018). Thus, based on the results of the present study, grown BSFL in the PKM in combination with FFP with or without fructose is suggested in BSFL farming that is used in feed industry.

Interestingly, the supplementation of fructose in the substrat of BSFL decreased the crude fat of BSFL. The effects of fructose on fat content can be highly dependent on factors such as overall diet quality, total calorie intake, and metabolic characteristics (Tappy and Lê 2010). One potential mechanism by which fructose may decrease fat content is through its effects on insect hormone that plays a key role in regulating fat metabolism and energy balance (Arrese and Soulages 2010).

On the other hand, the addition of fructose significantly increased carbohydrate content, but 10% fructose in the PKM:FFP significantly decreased the BSFL carbohydrate content. The supplementation of a lower fructose concentration (5%) in the substrate (PKM:FFP) significantly improved the ash content of the BSFL, while the highest moisture content was in BSFL reared in PKM:FFP without fructose.

Conclusions

The present study clearly demonstrated that FFP resulted in the highest crude protein and carbohydrate content when combined with PKM and fructose. Calcium, potassium, and magnesium levels did not differ significantly in the defatted BSFL meal fed PKM, FFP, and their combination, with or without fructose. Adding more than 5% fructose to the combination of PKM and FFP resulted in significantly higher iron content compared to other groups. Using PKM as the substrate for BSFL resulted in high relative concentrations of unsaturated fatty acids and lauric acid (C12:0), palmitoleic acid (C16:1), and linolelaidic acid (C18:2n6t) in BSFL oil. Nevertheless, docosadienoic acid was not detected in BSFL fed PKM. Minerals from PKM and FFP substrates can be converted significantly by BSFL, resulting in high amounts of minerals in the defatted BSFL meal. The BSFL reared in PKM with FFP supplemented with fructose had better MR but lower BCR. The survival rate of the BSFL did not differ significantly based on type of substrate. It may be advantageous/suggestion to ferment the PKM to potentially improve BSFL more efficiently and impart a high nutritional value. The PKM alternatively can be used as substrat for BSFL as FFP might have an expensive price in comparison to PKM. Furthermore, the mechanism by which fructose affects the growth, lipid metabolism, biomaterial reduction, and bioconversion of BSFL needs to be evaluated in future studies. The addition of more iron in the substrate of the BSFL might be having a good implementation of BSFL in the industrial level.

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Declarations

Conflicts of interest The authors declare no conflict of interest.

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