Available online at www.rajournals.in

RA JOURNAL OF APPLIED RESEARCH
ISSN: 2394-6709
DOI:10.47191/rajar/v8i11.06
Volume: 08 Issue: 11 November-2022

International Open Access

Impact Factor- 7.108

Page no.- 841-846

Proximate and Fatty Acid Profile Comparison of Black Soldier Fly Larvae Reared on Palm Kernel Meal and Cow Manure

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ARTICLE INFO

Published Online:
30 November 2022

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ABSTRACT

Black soldier fly (BSF) is becoming popular in the last few years. Several types of organic substrate have been tested as substrate for BSF larvae (BSFL) and resulted in valuable nutritional content of BSFL. The purpose of this study was to compare the nutritional content, fatty acid profile, and growth profile of BSFL grown between fermented Palm Kernel Meal (PKM) and fermented cow manure (CM). In total 3000 g of 7-day old larva (initial weight 0.044 g; length 12.44 mm) of BSF was randomly divided into two groups of triplicates. First group of BSFL was fed fermented PKM and second groups was fermented CM. After 15 days, BSFL was harvested, cleaned, dried, and separated between meal and oil. The BSFL meal was analyzed for proximate (Moisture, ash, crude protein, crude lipid, and carbohydrate), while oil for lipid profile. The growth of BSFL from two groups was determined by measuring 20 samples of BSFL per replication. Present study revealed that BSFL reared at fermented PKM had significantly higher crude protein (47.34%), ash (13.18%), lauric acid, heptadecanoic acid, total unsaturated fatty acid than fermented CM, but lower moisture (1.83%), crude lipid (4.31%), carbohydrate (33.34%), palmitoleic acid, myristoleic acid, linolenic acid, and arachidic acid. Linolelaidic acid and weight of BSFL grown in either fermented palm kernel meal or cow manure had no significantly difference. Present study suggested that both fermented PKM and CM can be used as substrate for growing BSFL that can convert into useful nutritional value.

KEYWORDS: Cow manure, Larvae, Palm oil, Nutritional value

INTRODUCTION

Black Soldier Fly/BSF (*Hermetia illucens*) is part of the insect class which is spread throughout the subtropical and tropical regions of the world (Alagappan et. al., 2022; Amrul et. al., 2022; Khairuddin et. al., 2022). The BSF has a larger size than most other flies with a length of about 15-20 mm, black and transparent in the abdominal segment (Amrul et. al., 2022; Oliveira et. al., 2015). The larvae of BSF/BSFL has been known as a substitute for animal feed among fish, chicken, or even bird breeders Heuel et. al. [5]; (Katya et. al., 2017; Patterson et. al., 2021). Several study informed that BSFL reduces the cost of large animal feed production (Al-Qazzaz et. al., 2016) and has a fairly high protein content of about 40% and the BSFL prepupae has a capability to accumulate 58% of lipid in its body (Mohd-Noor et. al., 2017). Besides high protein content, BSFL also has benefits for the environment, as a decomposer of organic waste (Visvini et. al., 2022). The BSFL can be grew at organic waste such as food waste, vegetables waste, fruits waste (Fischer and Romano, 2021; Lalander et. al., 2019). One example of organic waste that is easy to obtain and cheap is palm oil cake waste and livestock manure such as cow manure.

Indonesia is the world's largest producer of crude palm oil. This plant was initially existed in 1911, and it has been rapidly developed since 1970. Sumatra and Kalimantan provinces have the most oil palm plantations in Indonesia (Hambali and Rivai, 2017). From the production of oil in palm oil industry produces palm oil waste. Palm oil meal waste is a by-product resulting from the chemical and mechanical extraction of palm oil in the form of palm kernel. The Palm oil meal waste is used as a compost, but other alternatives are needed so that palm oil meal waste can be utilized optimally. One of the uses of palm oil meal waste is as a substrate for growing BSFL. This because it contains less 10% water, 14-17% protein, 9.5-10% fat and 12-18% crude fiber (Indariyanti and Barades, 2018).

Meanwhile, cow manure has not been less utilized due to the lack of public knowledge about the management of cow manure into a more useful material. Recently the management of cow manure is only used as a compost. However, (Ao et. al., 2021) proved that livestock manure can be bioconverted into organic growing media by BSFL. Indri et. al. [16] added that BSF female only lays eggs with growing substrate that have the smell of aromatic compounds and organic waste as its attractants. This attractant is obtained from the fermentation process by adding water to organic waste or chemically by using EM4. The organic waste that can produce an attractive substance is fermentable organic waste such as palm kernel meal waste, and cow manure. The BSFL fed cow manure waste had a higher percentage of essential and non-essential amino acids than BSFL fed pig manure. The essential amino acids content in the BSFL fed cow manure was higher than pig manure, except for the content of threonine and tryptophan. Meanwhile, the non-essential amino acid content in maggot fed pig manure was lower than cow manure, namely cysteine, serine, and glutamic acid (Newton et. al., 2005). Previous research, by (Indariyanti and Barades, 2018) mentioned that the application of palm oil cake fermentation substrate produced BSFL with water content (15.81%); ash (12.59%;); crude fiber (12.32%); proteins (40.55%); fat (6.85%); and carbohydrates (11.89%). In addition, (Masir et. al., 2020) stated that, a mixture of tofu waste and chicken manure treated with 5; 10; 15 days of rearing had optimum growth on the 15th day of BSFL rearing with a length x width of 1.46 x 0.36 mm, proving that maggot can be grown on decomposed organic waste.

Furthermore, substrates, such as mussels, bread, fish and food waste were shown involved on the fatty acid content in the BSFL. Regardless of nutrition, the larval fat was mostly composed of lauric acid and other saturated fatty acids, which were discovered to be synthesized by the BSFL. The fatty acid profile of the BSFL was shown to be affected by both the fatty acid content of the substrate and the BSFL weight. In general, heavier BSFL have a larger percentage of saturated fatty acids and a lower percentage of unsaturated fatty acids like eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) (Ewald et. al., 2020).

Although several studies have been conducted to determine the proximate and fatty acid analysis of BSFL on the various substrates. However, limited research has been conducted on the comparison of proximate and fatty acid profile analysis of BSFL grown on palm kernel meal waste and cow manure fermentation. Therefore, this research was conducted to evaluate the comparison of proximate and fatty oil profile of BSFL reared on palm oil meal waste and cow manure fermentation for 15 days. Growth of the BSFL fed either PKM or CM was also measured at the end of the study.

MATERIALS AND METHODS

PKM and Cow Manure

Palm kernel meal waste was obtained from PT Manunggal Jaya, East Kalimantan, Indonesia, while Cow Manure was collected from PT Berkah Salama Jaya, Loa Janan, Kutai Kartanegara, East kalimantan Indonesia. All chemical was analysis grade and purchased from Sigma Aldrich, Inc.

BSFL preparation.

In total of 3000 gr (Average initial weight 0.044 ± 0.003 g; length 12.44 ± 0.26 mm) of 7-day old larva (dol) was purchased from the CV Ahasa Larval group, a local BSFL farmer in Samarinda City, East Kalimantan, Indonesia. The BSFL that was purchased was a second generation which fed fermented palm kernel meal. The BSFL was randomly divided into two groups (PKM and Cow manure) of triplicates and kept in the plastic box containing one kg of substrate per 500 g of 7-dol of BSFL. The plastic box was lid using screen insect net, and maintained temperatures at 28.5 °C.

BSFL Substrate

Fermented palm kernel meal (PKM) and cow manure (CM) were performed by mixing PKM/CM, water, EM4 and sugar. Each substrate was mixed properly and place in the boks with lid and fermented for 4 days to produce fermented PKM/CM. After 7 days, fermented PKM/CM was used as substrate for BSFL.

BSFL rearing and sampel preparation

In total 500g of BSFL (7-dol) was added to each kilogram of fermented PKM/CM with Three replications each type of substrate. BSFL in the plastic box was grown in a room Temperature and relative humidity during the 15 days of study. Both fermented PKM and CM were provided only once for 15 days. An amount of 250 mL of distilled water was sprayed over the substrate to avoid substrate to be dried. At the end of the day 15, BSFL were collected, cleaned and dried using oven at 60 °C for 48h. Dried BSFL was crushed and separated between meal (Defatted) and oil using oil extractor (RG-307, Jiangsu, China). The defatted BSFL

meal was analysis for proximate analysis, while BSFL oil was prepared for fatty acid analysis.

Proximate analysis.

Using the method defined by the Association of Official Analytical Chemists (AOAC), the BSFL meal (Defatted) from each group was then processed for proximate analysis, including crude protein, crude fat, ash, and moisture content. Carbohydrate was determined using by difference method.

Fatty acids composition.

The fatty acid content of BSFL oil was analyzed using gas chromatography-mass spectrometry (GC/MS) (Agilent Technologies, CA, USA). By comparing GC retention periods and fragmentation patterns to valid standards and reference spectra included in the National Institute of Standards and Technology (NIST) library-MS databases 05, 08, and 11, methyl esters of fatty acids were identified.

Growth of BSFL

To evaluate the growth of the BSFL fed fermented PKM or CM, in total 20 BSFL from each replication was measured for their weight (g) and length (mm). The growth and length of BSFL were measured using standard electronic balance and digital calliper.

Statistical analysis

Data (weight, length, proximate and fatty acid composition) was presented as mean ± standard error (SE). To evaluate significance difference between mean, T-test analysis was performed using SPSS 22 (SPSS, Inc., USA). Mann Whitney test was done to determine significantly difference at $P<0.05$ between group treatment.

RESULTS AND DISCUSSION

The black soldier fly larvae (BSFL) comprises about 50% protein with a greater biological value than soybean protein (Shumo et. al., 2019). The BSFL nutrition value have been tested in several substrate, but not with fermented palm kernel meal and cow manure. Present study showed that BSFL reared at fermented Palm Kernel Meal had significantly higher crude protein (47.34%) and ash (13.18%) content than fermented cow manure, but lower moisture (1.83%), crude lipid (4.31%), and carbohydrate (33.34%) content (Table 1). This finding is similar to the previous study that the crude protein level (42.74%) of BSFL fed wheat bran group was greatest and lowest in the maize straw group (41.76%). The crude fat (30.55%) content of BSFL grown in maize straw was much lower than that of wheat bran (34.26%) (Gao et. al., 2019). According to Azizi et. al. [22], palm kernel cake (PKC) comprises 14-18% crude protein, 12-20% crude fiber, and other minerals. Furthermore, Balandrán-Quintana et. al. [23] reported that PKM has a protein value ranging from 14.4 to 20%, as well as a large quantity of carbs (50.3%) and crude fiber (16.7%). This can be a signed that BSFL has a good capability to convert palm kernel meal into valuable nutritional in BSFL body.

Table 1. Nutritional value of black soldier fly larvae (BSFL) fed fermented Palm Kernel Meal (PKM) and fermented cow manure (CM) for 15 days

Proximate (%)	Fermented Palm Kernel Meal	Fermented Cow Manure
Moisture	1.83 ± 0.02^a	2.30 ± 0.04^b
Ash	13.18 ± 0.04^a	11.14 ± 0.04^b
Crude Lipid	4.31 ± 0.02^a	4.47 ± 0.02^b
Crude Protein	47.34 ± 0.04^a	46.64 ± 0.04^b
Carbohydrate	33.34 ± 0.04^a	35.45 ± 0.06^b

Note: Mean ±Standard error followed by different alphabet superscript in the same row, is considered as significantly different at $P<0.05$.

Meanwhile, BSFL fed fermented palm kernel meal had a high lauric acid and heptadecanoic acid, but low in palmitoleic acid, myristoleic acid, linolenic acid, and arachidic acid. Linolelaidic acid of BSFL grown in either fermented palm kernel meal or cow manure had no significantly difference (Table 2). Further, total unsaturated fatty acid of BSFL fed fermented PKM showed significantly higher than BSFL grown in fermented cow manure (Figure 1). Past study performed by Gao et. al. [21], revealed that in comparison to BSFL collected from the wheat bran substrate, the lipid of BSFL had a higher proportion of monounsaturated fatty acid (24.86%) and polyunsaturated fatty acid (25.37%), but a lower proportion of saturated fatty acid (45.41%). Maize straw had a dry matter reduction of 48.41%. The fermented PKM may contain

Table 2. Fatty acid content of black soldier fly larvae (BSFL) fed fermented Palm Kernel Meal (PKM) and fermented cow manure (CM) for 15 days

Fatty acid content (%)	Fermented Palm Kernel Meal	Fermented Cow Manure
Lauric Acid	29.27 ± 0.19^a	23.00 ± 0.06^b
Myristoleic Acid	5.19 ± 0.07^a	7.15 ± 0.03^b
Palmitoleic Acid	15.81 ± 0.10^a	17.59 ± 0.10^b
Lenolelaidic_Acid	20.54 ± 0.16^a	20.08 ± 0.03^a
Linolenic Acid	1.85 ± 0.04^a	4.52 ± 0.16^b
Arachidic Acid	3.28 ± 0.09^a	10.18 ± 0.09^b
Heptadecanoic Acid	22.10 ± 0.10^a	17.00 ± 0.13^b

Note: Mean ±Standard error followed by different alphabet superscript in the same row, is considered as significantly different at $P<0.05$.

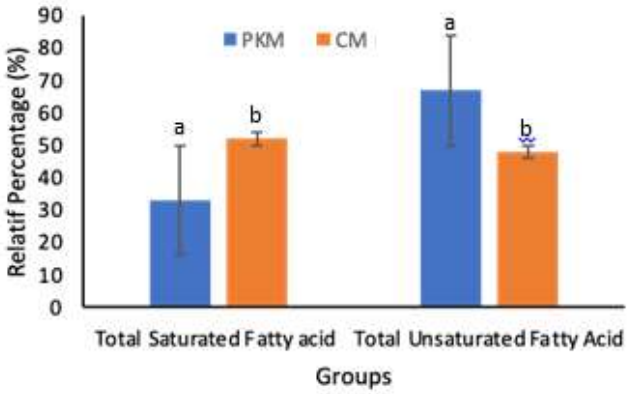

Figure 1. Fatty acid relatif concentration (%) of black soldier fly larvae (BSFL) fed fermented Palm Kernel Meal (PKM) and fermented cow manure (CM) for 15 days. Different alphabet above the bar at the same group, is considered as significantly different at *P<0.05*.

Furthermore, the BSFL may ingest any type of organic substrate, which is usually a waste product. The nutritional value of the substrates may influence on the growth of BSFL (Fischer and Romano, 2021). Based on present finding, the growth of BSFL fed either fermented PKM or fermented CM had no significantly difference, but length of the BSFL was affected by substrates (Figure 2). This may be that the nutritional content of the fermented PKM and fermented CM can be optimized by BSFL, resulting the similar weight parameter. Similar to past study that the weight of BSFL observed no significantly different when they were reared in the various substrate such as animal feed, food waste, and manure (Fitriana et. al., 2022).

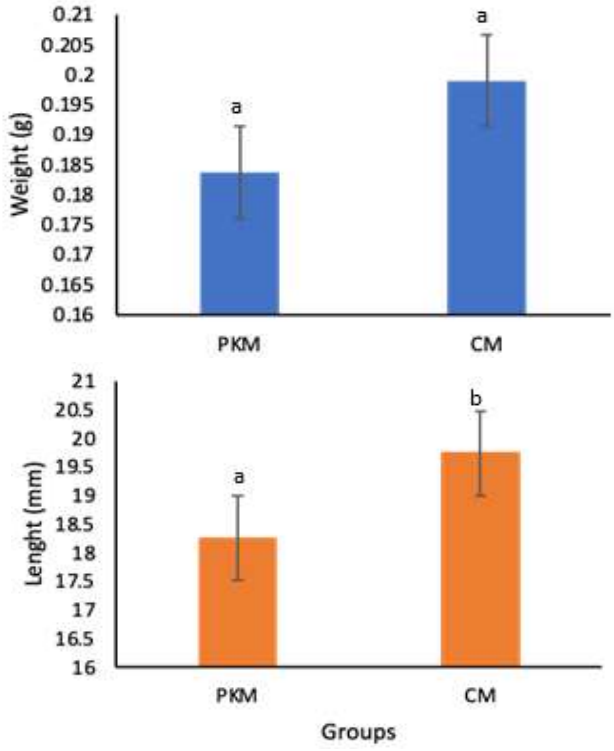

Figure 2. Weight and length of black soldier fly larvae (BSFL) fed fermented Palm Kernel Meal (PKM) and fermented cow manure (CM) for 15 days. Different alphabet above the bar at the same group, is considered as significantly different at *P<0.05*.

CONCLUSION

Either fermented Palm Kernel meal or fermented Cow Manure substrates had distinct benefits in terms of BSFL nutritional value and growth. The fermented PKM resulted in the highest crude protein, ash, lauric acid, heptadecanoic acid, total unsaturated fatty acid than fermented CM, although low in the moisture, crude lipid, carbohydrate, palmitoleic acid, myristoleic acid, linolenic acid, and arachidic acid. Linolelaidic acid and weight of BSFL grown in either fermented palm kernel meal or cow manure had no significantly difference. As a result, it may be advantageous to employ a combination of these substances to potentially generate BSFL more effectively and with a high nutritious value. It may be especially useful to investigate the mineral level, digestive enzyme activity and lipid gene metabolism of BSFL fed both fermented palm kernel meal and fermented cow manure.

ACKNOWLEDGEMENT

All author thankful to kedaireka matching fund financial year 2022, for funding. Grateful also extend to Department of Biology faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, East Kalimantan, Indonesia.

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