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Fatty acid composition of black soldier fly maggot were reared in the mixture of laying hen manure with lemuru fish oil

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Abstract. Manure can release greenhouse gases and invite the presence of house flies which can harm livestock health and pollute the environment. Maggot black soldier fly can overcome manure problems by converting it into nutrient-rich biomass. The growth and nutrient composition of maggot is influenced by the type of media used as food. In this study, the maggot black soldier fly was given six kinds of feed consisting of a mixture of laying hens manure with the addition of lemuru fish oil which varied 0%, 3%, 6%, 9%, 12%, and 15%. Three weeks old maggot black soldier fly was harvested and the total production was weighed. Maggot meal samples were analyzed for nutrient content and fatty acid composition. The data obtained were analyzed of variance and continued with Duncan's multiple range test. The results showed that the treatment was highly significant effect on maggot production, weight of 100 maggots, and crude fat content. Mixed feed of laying 61723 ASDBEENhens manure with 12% lemuru fish oil was the best mixture with the highest average production of fresh BSF maggot 1.139.25 g, average maggot weight 0.19 g/maggot, crude fat content 33.87%, linoleic 6.483%, linolenic acid 0.388%, EPA 2.142 %, and DHA 0.049%. It was concluded that the addition of lemuru fish oil into layer chicken manure could enrich the unsaturated fatty acid content of maggot black soldier fly.

Key words: BSF maggot, laying hens manure, fatty acid composition

1. Introduction

Fish meal is a feed ingredient that is widely used as a mixture of poultry rations. The availability of fish meal is feared to decrease so that it can cause price increases. Several researchers have tried to replace fish meal with maggot meal [1-8]. When compared to its nutrient content, fish meal contains higher protein and quite high essential fatty acids, while BSF maggot meal contains high crude fat but low essential fatty acid content.

The larvae of *Hermetia illucens* or better known as the maggot black soldier fly (BSF) are very well used as feed ingredients for protein sources to replace fish meal which has recently



been imported. Maggot BSF contains about 42% protein with 35% fat content [9]. The nutrient content in BSF maggots depends on the substrate used for growth, which generally uses waste, such as food waste [10], poultry manure [9,5], dairy cow manure [11].

Although chicken manure is suitable for reared BSF maggots, chicken manure actually lacks fat, according to Ghaly and MacDonald [12] laying hens manure contain 6.3% fat, 42.2% crude protein, 6.5% crude fiber and 33% carbohydrates. Slightly different from that reported by Shumo et al. [13] Chicken manure contains 2.7% fat, 15.3% crude protein, 18.3% ADF, 35.5% NDF and 86.6% organic matter. In fact, BSF maggots need a medium with a high fat content to be deposited in the body. The addition of fat or oil may help the growth of BSF larvae. Moreover, when using lemuru fish oil, it can be estimated that the BSF maggot produced will contain fat with a higher poly unsaturated fatty acid composition. But the price per kg of maggot is also higher.

Unsaturated fatty acids are needed by the human body because they can increase intelligence in children and prevent coronary heart disease in adults [26]. Therefore, livestock products such as meat and eggs should be cultivated to contain more unsaturated fatty acids such as omega-3 and omega-6. The content of unsaturated fatty acids in meat and eggs depends on the feed ingredients used for animal feed. Likewise, the fatty acid profile of BSF maggots is highly dependent on the substrate used. Ewald et al. [10] concluded that the fatty acid composition of BSF larvae was influenced by substrate feed and larval weight [42].

The amino acid profile of BSF maggot meal is similar to the amino acid profile of fish meal [14], but its fatty acid profile is not widely known. Ewald et al. [10] reported that BSF larvae contain high levels of saturated fatty acids 21% to 76%, monounsaturated fatty acids 32% and polyunsaturated fatty acids 23%. Previous studies have shown that it is possible to modify the fatty acid composition of BSF maggots. Knowledge of this fatty acid profile is very important so that the quality of BSF maggot is relatively the same as fish meal and can be used as animal feed ingredients. Thus, the aim of this study was to investigate how chicken manure mixed with various levels of lemuru fish oil affected the fatty acid composition of BSF biomass and BSF maggot production.

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2. Material and Method

2.1. Materials

The materials used in this experiment included laying hens manure, lemuru fish oil and BSF fly eggs. The tools used include 24 bioconverters, scales, dryers, and equipment for nutrient content analysis.

2.2. Research procedure

(1) Egg Collection

The collection of BSF fly eggs is carried out in closed cages, so that BSF flies come to deposit their eggs using fermented palm kernel meal. BSF fly eggs obtained were collected in a petri dish.

(2) Implementation of bioconversion

There were 24 bioconverters each filled with 2 kg of chicken manure, then lemuru fish oil was added according to treatment (0, 3, 6, 9, 12, and 15% of the weight of chicken manure). Mix until homogeneous. 0.1 g of BSF fly eggs were placed on chicken manure. After hatching, 2 kg of chicken manure and lemuru fish oil were added, then repeated in the second and third weeks.

(3) Harvesting maggot

Harvesting of BSF maggots was carried out at the age of 3 weeks. The BSF maggots that have been collected are then weighed and dried at a temperature of 50 °C.

(4) Nutrient Content Analysis

Samples of laying hens manure and BSF maggot were analyzed proximately.

(5) Fat extraction and Fatty acid analysis

BSF maggots were mashed, then fat extraction was carried out using the Bligh and Dyer [15] analysis method, while for fatty acid analysis using the analytical procedure of Christopher and Glass [16] with slight modifications.

2.3. Variable Response

The independent variable in this study was the level of addition of lemuru fish oil in laying hens manure as a growth medium for BSF maggots. The dependent variables were maggot production, weight of 100 maggots, nutrient content (protein, carbohydrates, fat, crude fiber, calcium, phosphorus, fatty acids, and gross energy) and fatty acid composition.

2.4. Statistical Analysis

This research is a pure experiment using Completely Randomized Design (CRD) with six treatments and four repetitions. In detail the six treatments were: M1: lemuru fish oil (0%); M2: 3% lemuru fish oil; M3: 6% lemuru fish oil; M4: 9% lemuru fish oil; M5: lemuru fish oil 12%; and M6: 15% lemuru fish oil. To determine the effect of treatment, statistical analysis was carried out the F test, if there was a significant effect, then to determine differences in the levels of lemuru fish oil used, Duncan's multiple range test was carried out [17].

3. Results and Discussion

3.1. Maggot Production

The highest average BSF maggot production of 1,139.25 g was obtained in the treatment using 12% lemuru fish oil mixing level (M5), and the lowest was 537.0 g in the treatment without using lemuru fish oil (M1) (Table 1). The results of the analysis of variance on the resulting maggot production data showed that the treatment had a highly significant effect ($P < 0.01$) on the fresh maggot production produced and the results of Duncan's multiple range test showed that the treatment of mixing lemuru fish oil was significantly different from the treatment without using lemuru fish oil, the use of Lemuru fish oil resulted in higher BSF maggot production, but the best mixing rate was 12%. Levels of mixing lemuru fish oil 3, 6, 9, and 15% gave relatively the same larval production.

Table 1. Average production of fresh BSF maggots (g) and weight of 100 fresh BSF maggots based on the level of treatment of mixing lemuru fish oil with laying hens manure

Treatment	Average production of larvae (g ± SEM)	Average weight of 100 fresh BSF larvae (g ± SEM)
M1 (0%)	537.00 ± 15.30 ^a	13.0150 ± 0.80 ^a
M2 (3%)	869.50 ± 36.34 ^b	15.4200 ± 0.19 ^{ab}
M3 (6%)	871.50 ± 44.08 ^b	14.4875 ± 0.71 ^a
M4 (9%)	852.75 ± 70.24 ^b	17.8400 ± 2.05 ^{bc}
M5 (12%)	1,139.25 ± 90.10 ^c	18.8625 ± 0.83 ^c
M6 (15%)	807.75 ± 57.99 ^b	19.3275 ± 0.79 ^c

Distinct superscript letters in the column indicate significant differences according to Duncan's test ($P \leq 0.05$).

The difference in the average BSF maggot production between the mixing level of 0% and 3-12%, is due to the maggot needing oil for growth and food reserves during the pupal and adult phases, by consuming lemuru fish oil and the protein contained in laying hens manure causes the maggot produced more. According to Sheppard *et al.* [9] larvae of black soldier flies contain 35% fat and 42% protein, while the results of research by Ewald *et al.* [10] black soldier fly larvae reared in food waste contain 40.7% fat and 36.6% protein so it needs to be provide in the media as a place of growth.

The mixing level of 15% lemuru fish oil gives lower yields compared to the mixing rate of 3-12%, this is thought to be less favored by the larvae because the media produced from this mixture is too sticky and smells fishy, which may interfere with the movement of the larvae.

The level of BSF maggot production in the media obtained in this study was relatively high, when converted it reached 7-14%. Almost the same as that reported by Lalander *et al.* [18] the maggot black soldier fly was able to convert 13.9% of food waste and 7.1% of poultry manure. This high level of BSF maggot production is thought to be caused by the manure used for BSF maggot growth media is still fresh, so that the available nutrients are sufficient to support maggot growth. According to Morgan and Eby (1975) in Tomberlin *et al.* [19] larvae of other diptera species such as house flies require fresh manure every day to support optimal growth. Manure that has been in the cage for a long time may have been eaten by house fly maggots and has been digested by anaerobic organisms, resulting in less available nutrients, thereby suppressing the growth of BSF larvae. Larrain and Salas [20] in their study used fresh manure less than 5 hours after being excreted by livestock.

3.2. Weight of 100 Maggots

The highest average weight of 100 fresh BSF maggots (Table 1) was obtained in the treatment using 15% lemuru fish oil mixing (M6) which was 19.3275 g, and the lowest was in the treatment without lemuru fish oil mixing (M1) which was 13.0150 g. The results of the analysis of variance on the weight data of 100 BSF maggots showed that the treatment had an effect on the weight of 100 fresh maggots. The higher the level of mixing of lemuru fish oil tends to produce heavier maggots. This is easy to understand because maggot really needs fat/oil to support its life development.

Duncan's test results showed that between M1, M2, and M3 did not show a significant difference, as well as between M4, M5, and M6 each produced almost the same maggot weight, so it can be said that the mixing rate of 12% is the mixing level of lemuru fish oil with optimal laying hens manure.

The M6 treatment produced the largest BSF maggot weight, but in terms of total fresh maggot production it was still below the M5 treatment, this may be due to the high mortality rate of larvae at a young age, as a result of the media being too sticky from this mixing.

The BSF maggot weight obtained from the results of this study ranged from 0.1301 g/maggot on laying hens manure media without lemuru fish oil, up to 0.1933 g/maggot on 15% lemuru fish oil mixing media. The average weight of BSF maggots obtained from the results of this study is slightly different (the resulting weight range is 0.1301-0.1933 g wider) with the results of research conducted by Tomberlin *et al.* [19] who got the weight range of black soldier larvae from 0.153-0.171 g/larvae. The difference in these results is due to the difference in the media used. While Nguyen *et al.* [21] obtained a larval weight of 339 mg/3 larvae or 0.113 g/larvae cultured in pig manure and Ewald *et al.* [10] obtained a larval weight of 0.191 g/larvae cultured in food waste.

3.3. Nutrient Content

The average nutrient content of BSF maggot meal based on the treatments is presented in Table 2. The nutrient content between treatments was quite varied, but statistically analyzed only crude fat content, because this nutrient was closely related to the treatment given.

Table 2. Nutrient Content of meal BSF larvae (% dry matter) based treatment

Nutrient	Treatment					
	M1	M2	M3	M4	M5	M6
Water (%)	3.60	2.34	3.49	3.41	3.46	3.95
Moisture (%)	17.20	13.80	15.63	15.10	12.96	13.30
Crude protein (%)	36.82	40.66	41.63	42.46	43.17	44.26
Crude fiber (%)	2.69	2.90	2.56	2.50	2.75	2.50
Crude fat (%)	24.80	26.22	28.15	32.23	33.87	34.39
Calcium (%)	1.47	1.45	1.31	1.21	1.25	1.20
Phosphorus (%)	0.78	0.65	0.56	0.71	0.55	0.65
Gross energy (Kkal/kg)	4,214.75	4,595.00	5,265.75	5,409.50	5,585.00	5,931.75
nitrogen-free extract (%)	18.47	16.47	12.02	7.70	7.24	3.70

The highest average crude fat content was obtained in the treatment using 15% lemuru fish oil mixing with laying hens manure (M6), which was 34.39%, and the lowest was in the treatment without using lemuru fish oil on laying hens manure medium (M1), which was 24.80% (Table 2). The results of the analysis of variance on the crude fat content data showed that the treatment had a highly significant effect on the crude fat content of BSF maggot meal.

Table 3. Average crude fat content of BSF larvae meal (g) produced by treatment of mixing lemuru fish oil with laying hens manure

Treatment	Average crude fat content of BSF larvae meal (g)
M1 (0%)	24.80 ± 0.31 ^a
M2 (3%)	26.22 ± 0.35 ^b
M3 (6%)	28.15 ± 0.22 ^c
M4 (9%)	32.23 ± 0.14 ^d
M5 (12%)	33.87 ± 0.23 ^e
M6 (15%)	34.39 ± 0.62 ^e

Distinct superscript letters in the column indicate significant differences according to Duncan's test ($P \leq 0.05$)

The difference in the average crude fat content of BSF maggot meal is thought to be due to differences in the size of BSF maggots. BSF maggots produced from M6 media are larger than

M1 media. Larger maggots are generally able to store more fat in their bodies, so they contain higher crude fat than smaller BSF maggots.

The crude fat content of maggot BSF ranged from 24.80-34.39% (Table 3), almost the same as that reported by Shumo *et al.* [13]. The crude fat content of BSF maggot meal is on average 30.1% in chicken manure and 34.3% in kitchen waste. Thus, the crude fat content of BSF larvae meal is highly dependent on the media used for its growth.

3.4. Fatty Acid Profile

The fatty acid profile of BSF maggot meal based on the treatment is presented in Table 4. However, only the fatty acids that were considered essential were analyzed, namely linoleic, linolenic, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA).

Table 4. fatty acid profile (%) of BSF larvae meal based treatment

Fatty acid	Treatment					
	M1	M2	M3	M4	M5	M6
Miristic	6.202	6.347	7.376	7.642	7.929	7.081
Palmitic	11.230	14.163	19.675	19.545	20.673	17.436
Palmitoleic	5.580	7.808	6.869	5.904	6.067	4.749
Stearic	1.417	1.400	2.024	2.457	2.116	2.104
Oleic	16.781	17.989	19.500	18.283	17.806	14.225
Linoleic	5.765	5.562	6.649	7.068	6.483	6.682
Linolenic	0.244	0.378	0.396	0.507	0.388	0.586
EPA	0	0	2.110	1.904	2.142	1.784
DHA	0	0	0.168	0.092	0.049	0

Keterangan: EPA = eicosapentaenoic acid (20:5n-3)
DHA = docosahexaenoic acid (22:6n-3)

Table 5. Average fatty acid content of meal BSF larvae (%) produced by treatment of mixing lemuru fish oil with laying hens manure

Fatty acid	Treatment					
	M1	M2	M3	M4	M5	M6
Linoleic	5.765 ± 0.09 ^{ac}	5.562 ± 0.24 ^c	6.649 ± 0.45 ^{ab}	7.068 ± 0.02 ^b	6.483 ± 0.46 ^{ab}	6.682 ± 0.22 ^{ab}
Linolenic	0.244 ± 0.03	0.378 ± 0.04	0.396 ± 0.07	0.507 ± 0.11	0.388 ± 0.12	0.586 ± 0.05
EPA	0 ± 0.00 ^a	0 ± 0.00 ^a	2.110 ± 0.12 ^c	1.904 ± 0.07 ^{bc}	2.142 ± 0.09 ^c	1.784 ± 0.11 ^b
DHA	0 ± 0.00 ^a	0 ± 0.00 ^a	0.1685 ± 0.01 ^b	0.092 ± 0.05 ^{ab}	0.049 ± 0.05 ^a	0 ± 0.00 ^a

Distinct superscript letters in the column indicate significant differences according to Duncan's test ($P \leq 0.05$).

3.5. Linoleic, Linolenic Fatty Acids, EPA and DHA

Linoleic and linolenic fatty acids were found in all types of media, while eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were found in media containing lemuru fish oil. Ewald *et al.* [10] stated that there are great similarities between the fatty acid composition of larvae and substrates. Linoleic fatty acid content in this study ranged from 5.562-7.068% (Table 5), the percentage is less than the results of Barroso *et al.* [22] and Liland *et al.* [23], linoleic acid (C18:2) was found in high percentages of 12-24% in larvae fed fish and chicken feed, or wheat and brown algae. And almost the same as the research of Ewald *et al.* [10] of 2.6-12.5% in larvae fed bread, leftovers, fish and shellfish.

The results of analysis of variance showed that the treatment level of mixing lemuru fish oil with laying hens manure had a highly significant effect ($P < 0.01$) on the linoleic fatty acid, EPA

and DHA content of BSF maggot meal, but had no significant effect on the linolenic fatty acid content. This effect is the result of the addition of lemuru fish oil. The results of the Agricultural Technology laboratory analysis at Gadjah Mada University Yogyakarta showed that lemuru fish oil contained linoleic, linolenic, EPA and DHA fatty acids, respectively 10.564%, 1.021%, 5.106%, and 2.966%. The addition of lemuru fish oil in laying hens manure was actually utilized by larvae and stored in the form of body fat, so that BSF maggots contained more linoleic, linolenic, EPA, and DHA fatty acids than without the addition of lemuru fish oil in laying hens manure media.

The content of linolenic fatty acid between treatments had no significant effect, this was due to the small amount of linolenic fatty acid in lemuru fish oil, namely 1.021%, so that a small amount of BSF maggot could be stockpiled. The fatty acid content of EPA and DHA began to be seen in the M3 treatment, and until the M5 treatment the EPA content was not significantly different. The DHA content was very high in the M3 treatment, but was not detected in the M6 treatment. DHA was not detected in the addition of 15% lemuru fish oil, it is possible that the larvae are no longer eating and store a lot of energy in the form of saturated fatty acids, according to the opinion of Ewald *et al.* [10] more energy is stored in the form of unsaturated fatty acids because these fatty acids are less susceptible to oxidation than unsaturated fatty acids. Furthermore, it is said that when the larvae gain weight, the percentage of EPA and DHA decreases. Meanwhile Liu *et al.* [24] found a decrease in unsaturated fatty acids over time as the black soldier fly larva (BSFL) got older. EPA and DHA were not found in the M1 and M2 treatments. This is easy to understand because the substrate contains little or no EPA and DHA according to the opinion of Liland *et al.* [23] EPA (C20:5) and DHA (C22:6) were found in the larvae, most likely from the substrate.

4. Conclusions and Suggestions

4.1. Conclusion

Lemuru fish oil is very well used for mixed BSF maggot growth media. The best mixture of lemuru fish oil is 12% of the weight of asfed laying hens manure, because it produces the most BSF maggot production with a fairly high average maggot weight and contains linoleic, linolenic, EPA and DHA fatty acids which are needed for Alabio duck feed supplements.

4.2. Suggestion

For optimal maggot growth, it is necessary to pay attention to the fat and protein content of the media used. If the laying hens manure used contains low fat, it is better to add lemuru fish oil 12% of the weight of the laying hens manure. Laying hens manure used should be fresh manure so that the nutrients contained in it can be immediately consumed by BSF maggots, without being overhauled by microorganisms.

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