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Utilization of defatted meal and oil from black soldier fly larvae (BSFL) *Hermetia illucens* in Nile tilapia *Oreochromis niloticus*

Pemanfaatan tepung tanpa lemak dan minyak larva black soldier fly (BSFL) *Hermetia illucens* pada ikan nila *Oreochromis niloticus*

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ABSTRACT

Defatted meal and oil derived from black soldier fly larvae (BSFL) can be used as substitutes for fish meal and fish oil. This study evaluated the utilization of defatted BSFL meal and BSFL oil in Nile tilapia (*Oreochromis niloticus*), both separately and in combination. This study employed a completely randomized design (CRD) with four treatments and three replications: control (without BSFL), TB (substitution of fish meal with defatted BSFL meal), MB (substitution of fish oil with BSFL oil), and TBMB (substitution of both fish meal and fish oil with defatted BSFL meal and BSFL oil). The substitution of defatted BSFL meal and BSFL oil showed no significant differences (P>0.05) between each treatment in growth performance, liver performance, digestibility, cholesterol, HDL, and LDL. However, in the TBMB treatment, there was a significant difference (P<0.05) compared to other treatments in triglycerides, FIFO ratio, and eFIFO ratio. The results indicate that defatted BSFL meal and BSFL oil can replace fish meal and fish oil without negatively affecting the growth performance of Nile tilapia while improving FIFO and eFIFO sustainability indicators.

Keywords: BSFL oil, defatted BSFL, FIFO, sustainable

ABSTRAK

Tepung BSFL tanpa lemak dan minyak BSFL dapat digunakan sebagai substitusi tepung ikan dan minyak ikan. Penelitian ini bertujuan untuk mengevaluasi pemanfaatan tepung BSFL tanpa lemak dan minyak BSFL pada ikan nila, baik secara terpisah maupun dalam kombinasi. Penelitian ini menggunakan rancangan acak lengkap (RAL) dengan empat perlakuan dan tiga ulangan, yaitu kontrol (tanpa BSFL), TB (penggantian tepung ikan dengan tepung BSFL tanpa lemak), MB (penggantian minyak ikan dengan minyak BSFL), dan TBMB (penggantian tepung ikan dan minyak ikan dengan tepung BSFL tanpa lemak dan minyak BSFL). Substitusi tepung BSFL tanpa lemak dan minyak BSFL tidak menunjukkan perbedaan signifikan (P>0,05) antarperlakuan pada kinerja pertumbuhan, kinerja hati, kecernaan, kolesterol, HDL, dan LDL. Namun, pada perlakuan TBMB terdapat perbedaan signifikan (P<0,05) dibandingkan dengan perlakuan lainnya pada trigliserida, rasio FIFO, dan rasio eFIFO. Hasil uji substitusi tepung BSFL tanpa lemak dan minyak BSFL dapat memberikan performa kinerja pertumbuhan ikan nila yang sama dengan pakan yang menggunakan tepung ikan dan minyak ikan serta memiliki rasio FIFO dan eFIFO yang mengindikasikan pakan ikan yang berkelanjutan.

Kata kunci: berkelanjutan, FIFO, minyak BSFL, tepung BSFL tanpa lemak

INTRODUCTION

Tilapia *(Oreochromis niloticus)* is a freshwater commodity widely cultivated and serves as a source of animal protein for humans. Indonesia is among the world's leading tilapia-producing countries, with exports valued at 81.7 million USD and totaling 11 thousand tons in 2023 (KKP, 2025). The growing global demand for tilapia poses a challenge for Indonesia, as it seeks to meet it by boosting tilapia production. Increased production must be supported by quality feed. According to Glencross (2020), the quality of feed ingredients determines the quality of feed.

Fish meal and fish oil are commonly used as primary ingredients in aquafeed formulations (Hodar *et al.,* 2020). Nonetheless, due to their continued reliance on natural catches, fish meal and fish oil are being reevaluated (FAO, 2022). Additionally, fish meal and fish oil compete with other sectors, such as human consumption and the livestock industry, thereby hindering the sustainability of fish farming (Naylor *et al.,* 2021). The FIFO ratio (fish in, fish out) has become an indicator of sustainability (Naylor *et al.,* 2021). According to Jackson (2009), the FIFO ratio represents the amount of wild fish, in the form of fish meal and fish oil, used in feed for farmed fish production. Substituting alternative ingredients is necessary to reduce dependence on fish meal and fish oil in fish feed and to support sustainable aquaculture (Kaushik & Troell, 2010; Ekasari *et al.,* 2018).

Alternative ingredients used to replace fish meal and fish oil include plant-based sources, animal by-products, and insects (Arru *et al.,* 2019; Malcorps *et al.,* 2019; Montoya-Camacho *et al.,* 2019; Nakharuthai *et al.,* 2020; Palupi *et al.,* 2020; Hua, 2021). Insects offer many benefits as protein and lipid sources, have a good essential amino acid profile, are environmentally friendly, and can serve as a sustainable ingredient in aquafeed (Gasco *et al.,* 2018; Tran *et al.,* 2022). Black soldier fly larvae (BSFL), with the scientific name *Hermetia illucens*, are one of the insects with balanced nutrient content. BSFL can also be produced continuously and do not compete with human needs, since they are not for human consumption (Liland *et al.,* 2017; Fauzi & Sari, 2018; Foysal *et al.,* 2021).

According to Müller *et al.* (2017), Black soldier fly larvae (BSFL) can serve as a sustainable source of protein and lipids. BSFL serves as a bioconversion agent, converting organic waste into biomass abundant in protein and fat (Franco *et al.,* 2021). The nutritional profile of BSFL is influenced by its rearing substrate (Hopkins *et al.,* 2021). In a food waste substrate, the nutrients of BSFL consist of 38.43% protein, 35.69% lipid, and 10.40% ash. It contains amino acids (histidine, isoleucine, leucine, lysine, threonine, valine, methionine, cysteine, arginine, alanine, aspartic acid, glutamic acid, glycine, proline, serine, tryptophan, and tyrosine) and fatty acids (lauric, palmitic, α-linolenic acid, and oleic acid) that are needed by fish (Fitriana *et al.,* 2022; Mohan *et al.,* 2022).

The high lipid content of BSFL meal makes it susceptible to lipid oxidation during storage, complicating feed processing (Henry *et al.,* 2015; Dumas *et al.,* 2018). Therefore, defatting is an option for processing BSFL meals, reducing lipid content and increasing protein content (Zozo *et al.,* 2022). Defatting can be performed using chemical solvents (hexane solution) or mechanical separation (Gómez *et al.,* 2019; Saviane *et al.,* 2021). The separation yields defatted BSFL meal, which is high in protein, and BSFL oil, which is abundant in fatty acids (Henry *et al.,* 2015). Defatted BSFL meal has been widely utilized as an ingredient for fish feed and has been tested on several types of aquaculture biota, such as Japanese seabass *Lateolabrax japonicus* (Wang *et al.,* 2019), grass carp *Ctenopharyngodon idellus* (Lu *et al.,* 2020), rainbow trout *Oncorhynchus mykiss* (Caimi *et al.,* 2021), and tilapia (Kishawy *et al.,* 2022).

BSFL oil has been tested as a substitute for fish oil in tilapia feed (Bakar *et al.,* 2021), rainbow trout (Fawole *et al.,* 2021), and common carp *Cyprinus carpio* (Herawati *et al.,* 2023). Kishawy *et al.* (2022) assert that defatted BSFL meal does not adversely affect the growth rate, feed conversion ratio, protein utilization, or nutritional digestibility of tilapia. Additionally, BSFL prepupa oil provides the highest growth and digestibility, and improves meat quality by providing balanced fatty acids in tilapia (Bakar *et al.,* 2021). Recent research has identified the usage of defatted black soldier fly larvae meal and black soldier fly larvae oil as substitutes for fish meal and fish oil in the diet of barramundi *Lates calcarifer* (Hender *et al.,* 2021). Nonetheless, the combination of defatted BSFL meal and BSFL oil has not been investigated in tilapia. The research seeks to assess the use of defatted BSFL meal and BSFL oil as substitutes for fish meal and fish oil in tilapia feed.

MATERIALS AND METHODS

Experimental design and fish rearing

The research design was a completely randomized design with four treatments and three replicates. The treatments used were control (fish meal and fish oil), TB (100% defatted BSFL meal and fish oil), MB (fish meal and 25% BSFL oil), and TBMB (100% defatted BSFL meal and 25% BSFL oil). The doses applied were based on research by Bakar *et al.* (2021) and Kishawy *et al.* (2022). The tilapia used in this study was from local farmers in Ciampea, Bogor, West Java. A total of 180 fish were used, with an initial weight of 6.28 ± 0.02 g. Fish were then divided into 12 rearing units.

The fish were reared in a 60×50×50 cm^3 aquarium, maintained at a water level of 30 cm (90 L volume), with a stocking density of 15 fish per aquarium. Fish were reared for 60 days and fed thrice daily at 08:00 AM, 12:00 PM, and 04:00 PM at satiation. Each feeding was weighed to determine feed consumption. Fish feces were siphoned, and 30% of the water was changed each morning before feeding. Water quality measurements during rearing consisted of temperature, DO, pH, and total ammonia nitrogen (TAN). The range of water quality during rearing was 25.6-27.2°C, DO 4.5-5 mg/L, pH 6.8-7.5, and TAN 0.21-0.40 mg/L. Fish were sampled on days 30 and 60 for total biomass.

Experimental diet

The defatted BSFL meal and BSFL oil used in this study were commercial products available in the marketplace. The defatted BSFL meal used has a protein content of 57.94%, lipid content of 9.9%, and ash content of 12.72%. The test diet used in this study is formulated feed with a protein content of 30%, formulated to meet the needs of tilapia. The ingredients and proximate composition are shown in Table 1. The amino acid profile and fatty acids of test diets are shown in Table 2. Cr_2O_3 was added as an indicator in the feed used in the digestibility performance test.

Proximate, amino acid, and fatty acid profile analysis

The ingredients defatted BSFL meal, BSFL oil, fish meal, fish oil, and test feed were proximate analyzed, including measurement of moisture content, protein, fat, ash, crude fiber, and nitrogen-free extract (NFE), as well as amino acid and fatty acid profiles. Moisture content was analyzed by heating in an oven at 105-110°C. Protein analysis was performed by the Kjeldahl method, crude fiber analysis by the acid and weak base dissolution method, ash content analysis by the furnace heating method at 600°C, and feed lipid content analysis using the Soxhlet method (Watanabe, 1998). Analysis of Amino acid and fatty acid profiles was performed using HPLC and GC methods (AOAC, 2012). The collected test feed and fish feces were analyzed for chromium (Cr_2O_3) content using the Takeuchi method (Takeuchi, 1988). Proximate analysis was also conducted on the fish body at the beginning and end of rearing to determine its retention value.

Digestibility analysis

Chromium (Cr_2O_3) at a dose of 0.05% was added to the feed as an indicator to observe the digestibility performance of fish (Takeuchi, 1988). Fish feces were collected one hour after feeding for several days until sufficient for analysis. Total digestibility is calculated using the following formula (Takeuchi, 1988):

$$\%\ \mathrm{KT} = \frac{\%\mathrm{Cr_20_3\ in\ diet}}{\%\mathrm{Cr_20_3\ in\ feces}} \times 100$$

Nutrient digestibility (protein and lipids) was calculated using the following formula (Takeuchi, 1988) :

$$\%\mathrm{KN} = 1 - \left(\frac{\%\mathrm{Cr_20_3\ in\ diet}}{\%\mathrm{Cr_20_3\ in\ feces}} \times \frac{\%\mathrm{nutrient\ in\ feces}}{\%\mathrm{nutrient\ in\ diet}}\right) \times 100$$

Blood chemistry analysis

Blood chemistry analysis includes triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and total blood cholesterol (TKD). Measurements were taken at the end of maintenance. They were made using an enzymatic colorimetric test for cholesterol with lipid clearing factor, using the cholesterol liquid color kit from Human Merck.

Hepatosomatic index (HSI) and liver lipid content

Liver samples measuring HSI parameters were obtained from 10 fish per replicate, and liver lipid content was measured using the Folch method (Watanabe, 1998). HSI was calculated as the liver weight (g) divided by the fish's body weight (g), using the formula described by Lamichhane *et al.* (2020).

$$\text{HSI (\%)} = \frac{100 \times \text{weight of liver (g)}}{\text{weight of fish (g)}} \times 100$$

Survival rate (SR)

The survival rate (SR) of fish in this study was determined using the formula provided by Effendie (2002):

$$\text{SR (\%)} = \frac{N_t}{N_0} \times 100$$

Note:

SR = Survival rate (%)

N_t = Quantity of fish at the end of treatment (fish)

N_0 = Quantity of fish at the beginning of treatment (fish)

Table 1. Test feed formulations using defatted BSFL meal and BSFL oil.

Ingredients (%)	Treatment			
	Control[1]	TB[1]	MB[1]	TBMB[1]
Fish meal	10	0	10	0
Defatted BSFL meal	0	10	0	10
Fish oil	1	1	0.75	0.75
BSFL Oil	0	0	0.25	0.25
Soybean meal	23	21	23	21
Wheat Flour	23.51	25.52	23.51	25.52
Cassava meal	10	10	10	10
Meat bone meal	5	5	5	5
Pollard	18.55	18.55	18.55	18.55
Corn gluten meal	5	5	5	5
Palm oil	2.33	2.33	2.33	2.33
Lysine	0.5	0.5	0.5	0.5
Methionine	0.5	0.5	0.5	0.5
Vitamin mix	0.2	0.2	0.2	0.2
Mineral mix	0.2	0.2	0.2	0.2
Polymethylolcarbamide (PMC)	0.2	0.2	0.2	0.2
Antioxidant	0.01	0.01	0.01	0.01
Proximate composition				
Water (%)	7.43	7.42	7.49	7.52
Protein (%)	29.91	29.49	29.95	29.91
Lipid (%)	6.20	6.47	6.33	6.40
Crude Fiber (%)	2.22	2.56	2.38	2.39
Ash (%)	7.55	7.19	7.58	7.26
NFE[2] (%)	46.69	46.87	46.27	46.51
GE[3] (kcal/kg)	4191	4200	4188	4203
C/P[4] (kcal/g)	14.01	14.24	13.98	14.05

Note: [1]Treatment without substitution (control), substitution with 100% defatted BSFL meal (TB), substitution with 25% BSFL oil (MB), substitution with 100% defatted BSFL meal and 25% BSFL oil (TBMB). [2]Nitrogen free extract (NFE) = 100 - (water + protein + lipid + crude fiber + ash). [3]Gross energy (GE) composition based on protein = 5.64 kcal/g protein, lipid = 9.44 kcal/g fat, and NFE = 4.11 kcal/g carbohydrate (Watanabe, 1998). [4]C/P = calories/protein. BSFL = black soldier fly larvae.

Specific growth rate (SGR)

Measurement of the specific growth rate (SGR) of the test fish was calculated based on the equation proposed by Huissman (1987):

$$SGR = \left(\frac{Ln\ Wt - Ln\ W0}{t}\right) \times 100$$

Note:
SGR = Specific growth rate (%/day)
t = Rearing time (days)
W_t = Average individual weight at the end of rearing (g)
W_0 = Average individual weight at the beginning of rearing (g)

Table 2. The amino acid profile and fatty acids of test diets using defatted BSFL meal and BSFL oil.

Composition	Treatment			
	Control	TB	MB	TBMB
Essential amino acids				
Arginine (%)	1.64	1.50	1.46	1.39
Histidine (%)	0.55	0.46	0.55	0.62
Isoleucine (%)	1.05	0.93	0.95	1.01
Leucine (%)	2.15	1.90	1.97	1.85
Lysine (%)	3.64	3.55	3.70	3.37
Methionine (%)	0.66	0.51	0.46	0.53
Phenylalanine (%)	0.88	0.81	0.91	0.79
Threonine (%)	0.58	0.51	0.62	0.46
Valine (%)	1.70	1.42	1.51	1.39
ΣEAA[1]	12.85	11.59	12.13	11.41
IEAA[2]	0.90	0.80	0.84	0.81
Non-essential amino acids				
Alanine (%)	0.70	0.61	0.65	0.52
Aspartic acid (%)	4.16	3.66	4.13	3.62
Cysteine (%)	0.54	0.44	0.51	0.47
Glutamic acid (%)	5.87	5.49	5.72	5.40
Proline (%)	0.82	0.72	0.85	0.76
Serine (%)	1.52	1.38	1.46	1.30
Tyrosine (%)	1.57	1.36	1.40	1.48
Glycine (%)	1.40	1.27	1.50	1.16
ΣNEAA[3]	16.58	14.93	16.22	14.71
Fatty Acids				
Saturated				
Lauric acid (12:0)	0.34	3.29	4.1	14.1
Myristic acid (14:0)	1.95	3.65	2.95	5.18
Palmitic acid (16:0)	27.08	26.43	26.11	27.69
Stearic acid (18:0)	0.64	0.61	0.54	0.47
Monounsaturated				
Oleic acid (18:1n-9)	46.88	51.19	53.93	48.95
Polyunsaturated				
Linoleic (18:2n-6)	4.63	3.25	2.21	3.52
Linolenic (18:3n-3)	0.55	0.81	0.73	1.02

Note: [1] Sum essential amino acids. [2] The essential amino acid index. [3] Sum non-essential amino acids. BSFL = black soldier fly larvae.

Feed conversion ratio (FCR)

The feed conversion ratio (FCR) was measured using the formula Huissman (1987):

$$FCR = \frac{F}{(B_t - B_0 + B_m)}$$

Note:
FCR = Feed Conversion Ratio
F = Amount of feed given during rearing (g)
B_t = Fish biomass at the end of rearing (g)
B_0 = Fish biomass at the beginning of rearing (g)
Bm = Biomass of dead fish (g)

Protein efficiency ratio (PER)

The protein efficiency ratio (PER) can be determined using the formula provided by Watanabe (1998):

$$PER = \frac{B_t - B_0}{P_i} \times 100$$

Note:
PER = Protein efficiency ratio (%)
B_t = Fish biomass at the end of rearing (g)
B_0 = Fish biomass at the beginning of rearing (g)
P_i = Amount of protein fed to fish (g)

Protein and lipid retention

Protein Retention Value can be calculated using the formula (Watanabe *et al.*, 2001):

$$\%RP = \left(\frac{F - I}{P \text{ or } L}\right) \times 100$$

Note:
RP = Protein Retention%
F = Amount of fish body protein at the end of rearing (g)
I = Amount of fish body protein at the beginning of rearing (g)
P = Amount of protein consumed by fish during rearing (g)
L = Amount of lipid consumed by fish during rearing (g)

Fish in fish out ratio (FIFO)

The fish in fish out ratio (FIFO) estimates the amount of wild fish used in feed to produce farmed fish (Jackson, 2009). FIFO can be calculated using the following formula:

$$FIFO = \left(\frac{FM\ (\%)}{FmY} + \frac{FO\ (\%)}{FoY}\right) \times FCR$$

Note:
FIFO = Fish in fish out ratio
FM = Fish meal content in feed (%)
FO = Fish oil content in feed (%)
FCR = Feed conversion ratio
FmY = Fish meal from wild fish 22.5%
FoY = Fish oil from wild fish 5%

The eFIFO (economic fish in fish out) ratio is a method for calculating the FIFO ratio that emphasizes economic factors (Kok *et al.*, 2020). The eFIFO ratio can be computed using the formula provided by Glencross et al. (2024).

$$FIFO = eFCR \times \left(\left(FM(\%) \times \frac{EVm}{(Evm \times FmY + Evo \times FoY)}\right) + \left(FO(\%) \times \frac{EVo}{(Evm \times FmY + Evo \times FoY)}\right)\right)$$

$$eFCR = \frac{F}{B_t}$$

Note:
eFIFO = Economic fish in fish out ratio
eFCR = Economic feed conversion ratio
FM = Fish meal content in feed (%)
FO = Fish oil content in feed (%)
EVm = Fish meal Price (US Dollar)
EVo = Fish oil price (US Dollar)
FmY = Fish meal from wild fish 22.5%
FoY = Fish oil from wild fish 5%
F = Amount of feed given during rearing (g)
B_t = Fish biomass at the end of rearing (g)

Data analysis

The research data were processed and analyzed utilizing Microsoft Excel 2019 and SPSS version 16.0. The data were assessed for normality and homogeneity before analysis of variance (ANOVA). An analysis of variance at the 95% confidence interval was conducted to ascertain the genuine effect of therapy on the test parameters. When outcomes are significantly divergent, the Duncan test is used to determine the true difference between treatments.

RESULTS AND DISCUSSION

Result

Amino acid profile of *defatted BSFL meal and fatty acid of BSFL oil*

The essential amino acid (AAE) profile has different limiting amino acids in each ingredient. In fish meals, threonine is the limiting amino acid, while in BSFL meals, soybean meals, and meat and bone meals, lysine is the limiting amino acid (Table 3). BSFL oil has a high lauric acid value (12:0) of 23.29% compared to fish oil's 0.03%. The fatty acid profile of BSFL oil is shown in Table 4.

Digestibility

The digestibility of tilapia fed the test diets (control, TB, MB, and TBMB) for 60 days is presented in Table 5. There were no significant differences (P>0.05) between the total digestibility, protein digestibility, and lipid digestibility values.

Table 3. Amino acid profiles of fish meal, defatted BSFL meal, soybean meal, and meat bone meal.

Amino Acids	Defatted BSFL meal	Fish meal	Soybean Meal[1]	Meat Bone Meal[1]
Essential				
Arginine (%)	2.90	3.20	3.44	3.73
Histidine (%)	0.73	1.02	1.25	1.14
Isoleucine (%)	1.64	2.05	2.15	1.57
Leucine (%)	4.30	5.10	3.58	4.28
Lysine (%)	5.96	6.22	2.91	3.63
Methionine (%)	0.70	0.97	0.65	1.00
Phenylalanine (%)	1.01	2.38	2.37	1.84
Threonine (%)	0.71	0.95	1.83	1.79
Valine (%)	2.86	3.17	2.47	2.92
ΣEAA[2]	20.81	25.06	20.65	21.90
IEAA[3]	1.26	1.61	1.49	1.55
Non-essential				
Alanine (%)	0.85	1.03	2.02	4.11
Aspartic acid (%)	5.88	6.85	5.31	4.00
Cysteine (%)	0.65	0.83	0.75	0.60
Glutamic acid (%)	7.13	9.08	9.53	7.52
Proline (%)	0.90	1.16	n/a	n/a
Serine (%)	2.35	2.75	2.30	2.16
Tyrosine (%)	2.47	2.96	1.60	1.72
Glycine (%)	2.02	2.54	2.02	6.87
ΣNEAA[4]	22.25	27.20	23.53	26.98

Note: [1]Source: IAFFD (2024), [2]sum essential amino acids, [3]index essential amino acid index, [4]sum non-essential amino acids, n/a: data not available. BSFL = black soldier fly larvae.

Table 4. Fatty acid profile of BSFL oil and fish oil.

Fatty Acids	BSFL Oil	Fish Oil
Saturated		
Lauric acid (12:0)	23.29	0.03
Myristic acid (14:0)	9.52	1.78
Palmitic acid (16:0)	26.6	21.26
Stearic acid (18:0)	0.39	0.63
Monounsaturated		
Oleic acid (18:1n-9)	49.08	52.45
Polyunsaturated		
Linoleic (18:2n-6)	4.22	3.93
Linolenic (18:3n-3)	0.89	0.93

Note: BSFL = black soldier fly larvae.

Blood chemistry

Triglyceride levels exhibited significant differences (P<0.05). TBMB had the lowest triglyceride level at 62.44 mg/dL. The blood chemistry parameters of cholesterol, HDL, and LDL showed no difference (P>0.05).

Hepatosomatic index (HSI) and liver lipid

The hepatosomatic index (HSI) and liver lipid of fish fed with BSFL meal and BSFL oil substitutes showed no significant difference (P>0.05). Tilapia fed the TBMB test had a higher hepatosomatic index (HSI) of 2.75 than the other treatments. The hepatosomatic index (HSI) and liver lipids of tilapia are displayed in Table 7.

Growth performance

Growth performance is determined by several parameters, such as survival rate (SR), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein retention, and lipid retention. The substitution of defatted BSFL meal and BSFL oil did not significantly affect (P>0.05) the growth performance of tilapia raised for 60 days. The TBMB treatment showed the highest protein retention (37.05%) and the lowest lipid retention (29.89%). The growth performance of tilapia can be seen in Table 8.

Fish in fish out ratio

Fish in fish out ratio (FIFO) in tilapia fed with defatted BSFL meal substitution and BSFL oil

Table 5. Digestibility performance of tilapia fed with BSFL meal and BSFL oil substitutes.

Digestibility Performance	Treatment			
	Control	TB	MB	TBMB
Total Digestibility (%)	68.15 ± 1.86	66.66 ± 1.13	67.82 ± 1.08	66.49 ± 1.99
Protein Digestibility (%)	84.12 ± 1.74	84.73 ± 2.66	84.80 ± 2.46	85.07 ± 2.36
Lipid Digestibility (%)	86.23 ± 2.19	87.90 ± 2.33	88.15 ± 2.00	87.07 ± 2.48

Note: Digestibility performance did not differ significantly (P>0.05). The data displayed in the table represent the mean and standard deviation. Treatment without substitution (control), substitution with 100% defatted BSFL meal (TB), substitution with 25% BSFL oil (MB), substitution with 100% defatted BSFL meal and 25% BSFL oil (TBMB). BSFL = black soldier fly larvae.

Table 6. Blood chemistry of tilapia fed with BSFL meal and BSFL oil substitutes.

Blood Chemistry	Treatment			
	Control	TB	MB	TBMB
Triglycerides (mg/dL)	102.53 ± 7.93^{c}	82.19 ± 2.75^{b}	73.67 ± 4.40^{ab}	62.44 ± 7.38^{a}
Cholesterol (mg/dL)	110.87 ± 7.23^{a}	105.62 ± 7.96^{a}	102.28 ± 9.34^{a}	100.23 ± 5.53^{a}
HDL[1] (mg/dL)	54.33 ± 5.86^{a}	53.33 ± 9.50^{a}	57.33 ± 8.39^{a}	61.33 ± 5.03^{a}
LDL[2] (mg/dL)	47.00 ± 6.24^{a}	45.67 ± 7.51^{a}	43.67 ± 9.02^{a}	42.67 ± 3.51^{a}

Note: [1]HDL = high density lipoprotein, [2]LDL = low density lipoprotein. Values in the same column followed by the same letter are not significantly different (P>0.05). The data displayed in the table represent the mean and standard deviation. Treatment without substitution (control), substitution with 100% defatted BSFL meal (TB), substitution with 25% BSFL oil (MB), substitution with 100% defatted BSFL meal and 25% BSFL oil (TBMB). BSFL = black soldier fly larvae.

Table 7. Hepatosomatic index (HSI) and liver lipids of tilapia fed with BSFL meal and BSFL oil substitution diets.

Liver Performance	Treatment			
	Control	TB	MB	TBMB
HSI[1]	2.60 ± 0.25	2.62 ± 0.03	2.64 ± 0.23	2.75 ± 0.13
Liver lipids (%)	7.52 ± 1.25	6.72 ± 1.33	6.30 ± 1.32	6.31 ± 1.10

Note: [1]HSI: Hepatosomatic index and liver lipids showed no significant difference (P>0.05). The data displayed in the table represent the mean and standard deviation. Treatment without substitution (control), substitution with 100% defatted BSFL meal (TB), substitution with 25% BSFL oil (MB), substitution with 100% defatted BSFL meal and 25% BSFL oil (TBMB). BSFL = black soldier fly larvae.

in all treatments showed significant differences (P<0.05). The TBMB treatment exhibited the lowest value of 0.18, followed by the TB treatment at 0.25, the MB treatment at 0.73, and the control treatment at 0.79. FIFO values are shown in Figure 1. The eFIFO (economic fish in fish out) ratio in tilapia fed with BSFL substitution showed a significant difference (P<0.05). The eFIFO values are shown in Figure 2.

Discussion

Feed ingredient quality is evaluated based on protein bioavailability and amino acid composition (Audia *et al.,* 2026). Defatted BSFL meal contains a high protein concentration and an amino acid composition similar to a fish meal. Henry *et al.* (2015) state that BSF meal from the order Diptera has an amino acid profile similar to fish meal. Methionine remains the limiting amino acid in defatted BSFL meal, but the lysine value in defatted BSFL meal approaches the lysine value in fish meal (Table 3). Lysine and methionine are limiting amino acids in plant-based protein sources and by-products (NRC, 2011; Malcorps *et al.,* 2019).

Tilapia (*O. niloticus*) requires 1.3-1.44% lysine and 0.15-1.35% methionine (NRC, 2011). Amino acids play a crucial role in nutrient

Table 8. Growth performance of tilapia fed with BSFL meal and BSFL oil substitutes.

Growth Performance	Treatment			
	Control	TB	MB	TBMB
B0 (g)	94.23 ± 0.27	94.22 ± 0.28	94.20 ± 0.25	94.18 ± 0.20
Bt (g)	477.93 ± 9.96	479.47 ± 10.32	488.37 ± 10.08	491.73 ± 9.40
W0 (g)	6.28 ± 0.02	6.28 ± 0.02	6.28 ± 0.02	6.28 ± 0.01
Wt (g)	31.86 ± 0.66	31.96 ± 0.69	32.56 ± 0.67	32.78 ± 0.63
SR (%)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
SGR (%)	2.71 ± 0.03	2.71 ± 0.04	2.74 ± 0.04	2.75 ± 0.03
FI (g)	470.60 ± 9.67	474.63 ± 11.14	482.20 ± 10.15	485.13 ± 10.34
FCR	1.23 ± 0.03	1.23 ± 0.01	1.22 ± 0.02	1.22 ± 0.02
PER	2.73 ± 0.06	2.75 ± 0.02	2.73 ± 0.05	2.74 ± 0.04
RP (%)	34.62 ± 2.96	35.15 ± 3.15	35.26 ± 2.51	37.05 ± 2.70
RL (%)	34.00 ± 4.04	32.24 ± 4.18	31.41 ± 1.13	29.89 ± 7.91

Note: B0 = initial biomass weight, Bt = final biomass weight, W0 = average initial weight, Wt = average final weight, SR = survival rate, SGR = specific growth rate, FI = feed intake, FCR = feed conversion ratio, PER = protein efficiency ratio, RP = protein retention, RL = lipid retention, growth performance showed no significant difference (P>0.05). The data displayed in the table represent the mean and standard deviation. Treatment without substitution (control), substitution with 100% defatted BSFL meal (TB), substitution with 25% BSFL oil (MB), substitution with 100% defatted BSFL meal and 25% BSFL oil (TBMB). BSFL = black soldier fly larvae.

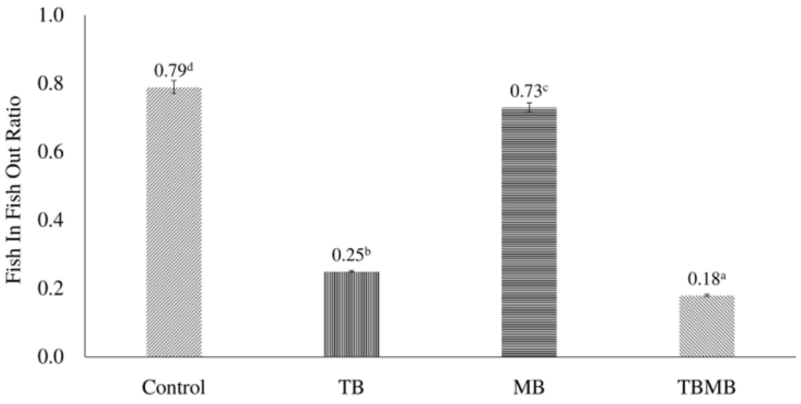

Figure 1. Fish in fish out ratio of tilapia fed with black soldier fly larvae (BSFL) meal and BSFL oil substitution diets. Distinct letters above the bar chart denote significant differences (Duncan's test: P<0.05). Treatment without substitution (control), substitution with 100% defatted BSFL meal (TB), substitution with 25% BSFL oil (MB), substitution with 100% defatted BSFL meal and 25% BSFL oil (TBMB).

metabolism, including protein synthesis (Church *et al.,* 2020), and their deficiency can trigger protein degradation (Cleveland & Radler, 2019). BSFL oil used as a lipid source in feed has lauric, palmitic, oleic, linoleic, and linolenic fatty acid values (Table 4). BSFL oil contains a high concentration of saturated fatty acids (SFA), including lauric and palmitic acids (Meneguz *et al.,* 2018; Ewald *et al.,* 2020; Bakar *et al.,* 2021). Lauric fatty acid is also classified as a medium-chain fatty acid (MCFA), with 12 carbon chains (Liland *et al.,* 2017). MCFA can be used as an energy source and minimize lipid accumulation in tissues (Li *et al.,* 2016; Belghit *et al.,* 2019).

Defatted meal and oil derived from BSFL possess potential as ingredients for aquaculture feed (Benzertiha *et al.,* 2020; Nairuti *et al.,* 2022). Defatted BSFL meal and BSFL oil did not influence the digestibility of tilapia (Table 5). These results were analogous to the research of Kishawy *et al.* (2022), who reported that defatted BSFL meal did not affect tilapia digestibility. Defatted BSFL meal did not elicit substantial alterations in digestive enzymes, trypsin, alpha-amylase, and lipase (Li *et al.,* 2017). In vitro digestibility of defatted BSFL meal was better than that of full fat BSFL meal (Traksele *et al.,* 2021).

BSFL oil did not affect nutrient digestibility (Benzertiha *et al.,* 2020). However, Bakar *et al.* (2021) reported that substitution of 50% and 100% BSFL prepupae oil gave poor lipid digestibility in tilapia due to too high SFA. Digestibility is one of the key indicators for the availability and sustainability of feed ingredients (Fontes *et al.,* 2019). There was a striking difference in triglyceride (TG) levels between the BSFL and the control treatments. Treatments with defatted BSFL meal and BSFL oil showed lower TG levels. Defatted BSFL meal has been shown to reduce TG levels in fish (Wang *et al.,* 2019).

According to Wardani *et al.* (2021), a decrease in TG in the blood indicates the utilization of non-protein energy. Cholesterol, HDL, and LDL levels in tilapia fed a diet containing a substitution of defatted BSFL meal and BSFL oil showed no significant difference. This result is the same as the research of Lu *et al.* (2020). Feeding with a defatted BSFL meal does not affect the cholesterol value of grass carp. Wang *et al.* (2019) stated that the use of defatted BSFL meal had no impact on cholesterol transport between peripheral tissues and the hepatopancreas. However, in the study of Li *et al.* (2017), defatted BSFL meal can significantly reduce cholesterol levels in fish. This disparity arises from variations in fish species, size, insect type, insect developmental stage, and insect processing methods (Tschirner & Simon, 2015).

This study showed that tilapia fed defatted BSFL meal and BSFL oil did not show significant differences in HSI or liver lipid values. These results align with Renna *et al.* (2017) and Fawole *et al.* (2020), who reported that the BSFL meal does not affect the HSI value. HSI value is one of the liver performance parameters commonly used to assess fish nutritional status quickly and easily (Liu *et al.,* 2018). Lauric acid content in the TBMB feed (Table 2) resulted in higher HSI values in the TBMB treatment and lower liver lipid content compared to other treatments (Table 7). Belghit *et al.* (2019) stated that high lauric acid in insect-based feed can reduce liver fat. The decrease in liver lipid levels indicates that

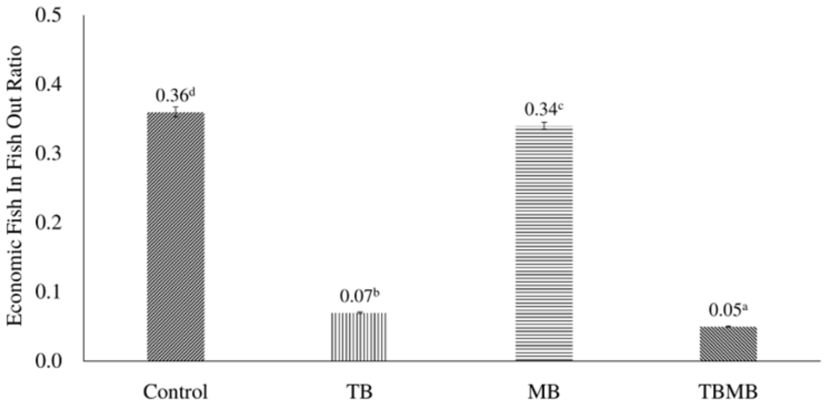

Figure 2. Economic fish in fish out ratio of tilapia fed with black soldier fly larvae (BSFL) meal and BSFL oil substitution diets. Distinct letters above the bar chart denote significant differences (Duncan's test: P<0.05). Treatment without substitution (control), substitution with 100% defatted BSFL meal (TB), substitution with 25% BSFL oil (MB), substitution with 100% defatted BSFL meal and 25% BSFL oil (TBMB).

some of the lipids are utilized as an energy source (Setiawati *et al.,* 2016).

The results of this study indicated that defatted BSFL meal and BSFL oil exhibited no significant differences (P>0.05) in any growth performance parameter (Table 8). These results are similar to several studies using defatted BSFL meal in tilapia with 100% substitution (Kishawy *et al.,* 2022), in rainbow trout with up to 50% substitution (Caimi *et al.,* 2021), in Japanese seabass with up to 64% substitution (Wang *et al.,* 2019), in vannamei shrimp with up to 60% (Wang *et al.,* 2021), and in grass carp with 100% substitution (Lu *et al.,* 2020). These similar results are due to the nutritional content of defatted BSFL meal not being significantly different from that of fish meal (Devic *et al.,* 2018). BSFL oil is reported to have no negative effects on growth, feed consumption, feed efficiency, or nutrient retention (Bakar *et al.,* 2021; Fawole *et al.,* 2021; Herawati *et al.,* 2023).

The combined use of defatted BSFL meal and BSFL oil replacement did not markedly influence growth. The results were similar to the research of Hender *et al.* (2021). Substitution of 30% BSFL meal and 30% defatted BSFL oil in the diet of barramundi does not affect growth. However, the study of Fawole *et al.* (2020) gave different results, with a 50% substitution of defatted BSFL meal in African catfish significantly affecting growth and nutrient utilization. Most fish can be fed BSFL-based diets, but results will vary depending on the fish species and the BSFL substrate (Hender *et al.,* 2021).

The protein retention value was highest in the TBMB treatment, while the lipid retention value was lowest compared with the other treatments (Table 8). This is due to the high MCFA content in the TBMB treatment. MCFA, such as lauric acid, is rapidly catabolized and used as energy, thereby reducing lipid accumulation in the body (Li *et al.,* 2016; Belghit *et al.,* 2019; Jadhav & Annapure, 2021). MCFA can enhance ketogenesis because it is more rapidly oxidized than LCFA (long-chain fatty acid), allowing the mitochondria to more readily access MCFA for β-oxidation, which produces energy (Luo *et al.,* 2014). Ketogenesis occurs in the liver, and one of its functions is nutrient metabolism (NRC, 2011; Xie *et al.,* 2021).

Significant differences were observed between interventions in the fish in fish out (FIFO) and economic fish in fish out (eFIFO) ratios of this study (Figures 1 and 2). In the research of Kok *et al.* (2020), freshwater fish such as tilapia, carp, and catfish have low FIFO values ranging from 0.06-0.15. The lowest FIFO and eFIFO ratios in this study were observed in the TBMB (FIFO 0.18 and eFIFO 0.05) and TB (FIFO 0.25 and eFIFO 0.07) treatments, indicating that substituting defatted BSFL meal can reduce the FIFO ratio in tilapia feed. This result is supported by Stejskal *et al.* (2020), who state that the FIFO ratio negatively correlates with the BSFL meal substitution level.

Research (Mikołajczak *et al.,* 2022) shows that substituting a 20% BSFL meal can decrease the FIFO ratio by 30%. The FIFO ratio is one aspect of sustainability for marine ecosystems. The FIFO and eFIFO ratios elucidate the utilization of wild fish as fish meal and fish oil in the production of farmed fish, with the eFIFO ratio delineating an economic allocation of these resources (Jackson, 2009; Glencross *et al.,* 2024). The eFIFO ratio also supports life cycle assessment (LCA) to examine environmental implications (Kok *et al.,* 2020).

Defatted BSFL meal is a sustainable ingredient that replaces marine ingredients in feed (Maulu *et al.,* 2022). Substitution of fish meal with BSFL meal can reduce global warming potential (GWP) by 31% (Mertenat *et al.,* 2019). In a study by Smárason *et al. (*2017), using BSFL in fish feed can reduce the carbon footprint compared to conventional feed. BSFL utilization can reduce environmental impacts and be applied in the circular economy (Smárason *et al.,* 2017; Zarantoniello *et al.,* 2020; Stejskal *et al.,* 2023).

CONCLUSION

Defatted BSFL meal and BSFL oil, utilized independently or in combination, can yield equivalent growth performance in tilapia as that achieved with fish meal and fish oil feed.

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