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Impact of black soldier fly (*Hermetia illucens*) fresh meal on the growth performance, digestive enzymes, hematology, and intestinal histology of cork fish (*Channa striata*)

Boby MUSLIMIN[1,4], Danang YONARTA[2,*], Eny HERIYATI[3], Helmizuryani HELMIZURYANI[4], Meika Puspita SARI[4], Dewi APRIYANTI[1], Rahmat KURNIAWAN[5]

[1]Research Center of Conservation for Marine and Inland Water Resources, National Research and Innovation Agency, Indonesia
[2]Aquaculture Study Program, Faculty of Agriculture, Sriwijaya University, Indralaya, South Sumatra, Indonesia
[3]Marine Science Study Program, Kutai Timur Agricultural College, East Kutai Regency, Indonesia
[4]Aquaculture Study Program, Faculty of Agriculture, Muhammadiyah University of Palembang, South Sumatra, Indonesia
[5]Agribusiness Study Program, Faculty of Agriculture, Muhammadiyah University of Palembang, South Sumatra, Indonesia

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Abstract: Black Soldier Fly (BSF) is an alternative ingredient for Fish Meal (FM) which is expected to reduce aquaculture operational costs and positively impact fish growth and health. The effect of BSF fresh meal on growth response, digestibility, intestinal hematology, and intestinal histology of cork fish was investigated. The BSF experiment with mixed percentages as treatments, by 20%, 50%, 80%, and 100%, was repeated four times. Furthermore, there were 50 individuals/m^3 cork fish with an average weight of 1.6 ± 0.05 g distributed in each treatment. An increase in BSF influences a significant decrease in the Relative Growth Weight (RGW) and Specific Growth Rate (SGR) while the Feed Conversion Ratio (FCR) value has improved. The enzymes were significantly increased, and the histological morphology of the intestine was normal. The highest leukocytes and erythrocytes reached 5.29 ± 1.08 × 10^4/mm^3 and 3.22 ± 1.37 × 10^6/mm^3, within the normal range and slightly above the threshold of 3.00 × 10^6/mm^3. Meanwhile, BSF fresh meal as a substitute feed recommended for cork fish is not more than 20%, which can impact better growth and digestibility.

Key words: Cork fish, substitute feed, digestibility, black soldier fly

1. Introduction

Global demand for fish is estimated to increase with the human population [1] and the fulfilment is carried out through catch and farming. Since there is a decrease in fish catches, food needs can be met by farming instead. According to Food and Agriculture Organization (FAO) [2], global data for farming has increased, while fish catch is stagnant and even predicted to decrease. In Indonesia, fish farming is targeted to grow by 8.5% per year [3] and its main carrying capacity is feed. The fish feed price has increased yearly, and alternative materials are needed to reduce production costs in the business. Maggot is the favourite feed ingredient used as feed due to its reduced organic waste.

According to the Indonesia's waste production in 2021 will reach 30.8 million t, where 40% is organic waste from food and the decomposition takes up to 12 months [4]. Maggot is larvae produced from the Black Soldier Fly (BSF) as the organic waste decomposer. BSF fresh meal has an excellent proximate content as a substitute for the fish meal (FM) as raw material for pellets, and the majority is obtained from imports every year. This is due to the protein content of the maggots nearby to the FM, i.e. 40.12% and 45.5% [5], [6].

Research on BSF as feed has been conducted on *Argyrosomus regius*, *Seriola quinqueradiata*, *Pelteobagrus fulvidraco*, and *Oreochromis niloticus* [7]–[10]. According to a meta-analysis [11], the response of fish growth to BSF was different. However, the suitability of physiology and fish health status in cork fish has not been evaluated and will be studied in this research. Independent feed production from organic waste can reduce organic waste and be converted into raw materials to replace pellets. This independent feed production is expected to increase sustainable and environmentally friendly aquaculture with blue economy value.

2. Materials and methods

2.1. Time and location

Fish rearing was carried out for 3 months, from February to May 2022. The experiment was conducted at the Independent Sumatran People's Hatchery Unit, South Sumatra.

* Correspondence: danangyonarta@unsri.ac.id

The digestive enzymes analysis parameter was carried out at the Dairy Animal Nutrition Laboratory, IPB University, and the hematological analysis was at the Department of Aquaculture, Sriwijaya University. Meanwhile, proximate analysis was performed at the Department of Fisheries Products, Sriwijaya University, and histological analysis was carried out at the Department of Aquaculture, IPB University.

2.2. Experimental procedure

The number of fish used was 50 individuals/m^3 with an average weight of 1.6 ± 0.05 g. They were reared in cement ponds with ad libitum feeding, and the amount of feed consumed was calculated. Furthermore, feeding is conducted in the morning, evening, and night. Experiments were using commercial Fish Meal with BSF percentages of 20 (B20), 50 (B50), 80 (B80), and 100 (B100). Treatment repetitions were carried out 4 times using a completely randomized design. The nutritional composition of commercial feed (PF500™) consists of 39% protein, 5% fat, 10% carbohydrates, and 11% ash content. Before being given to the fish, fresh BSF is dried by heating it gradually in the oven. The Fresh BSF with a length of 20–25 mm were acquired from breeders in Palembang, South Sumatra, Indonesia. The drying process began with the BSF being washed to remove residue, drained, spread on a tray lined with aluminum foil, and placed in a gradually heated oven. The temperature of the oven was gradually increased to 60 and 85 °C for 2 and 8 h, respectively. The dried BSF product and commercial feed were stored in jars after being weighed according to the treatment. At the end of the study, the amount of feed remaining in the jars was recorded.

Fish sampling was performed randomly with 30% of the total population. The sampling of growth in weight, length, and quality was carried out every 20 days. Measurement of weight growth used digital scales and length with calipers. Digestive enzyme analysis, hematology, and histology were conducted at the end of the study. Moreover, euthanasia was carried out for all three parameters by lowering the water temperature to 5 °C using ice gel.

Analysis of amylase and lipase as well as protease enzymes was conducted following the procedure of Natalia [12] and Afifah et al. [13]. The materials for amylase analysis were Tris buffer solution (20 mM Tris HCl, 1 mM EDTA, 10 mM $CaCl_2$, pH 7.5), starch solution (in 20 mM sodium phosphate pH 6.9), 6 mM NaCl, and dinitro salicylate solution. Tris buffer (20 mM Tris HCl, 1 mM EDTA, 10 mM $CaCl_2$, pH 7.5), Tris-HCl (0.1 M pH 8.0), virgin olive oil, 95 % ethyl alcohol, and NaOH 0.001 N were the materials for lipase analysis. Meanwhile, the materials for the protease test were Tris buffer (20 mM Tris HCl, 1 mM EDTA, 10 mM $CaCl_2$, pH 7.5), phosphate buffer (0.05 M, pH 7), casein substrate solution (pH 7), Tyrosine solution (5 mM/L), distilled water, TCA solution (0.1 M), $CaCl_2$ (2 mM/L), Na_2CO_3 (0.4 M), and Folin Ciaocalteau solution. These three enzymatic tests used Erlenmeyer, centrifugation, and a spectrophotometer.

Blood samples were collected into microtubes to observe white blood cells, red blood cells, hemoglobin, and hematocrit. The Leukocyte and Erythrocyte Test referred to [14]. The materials utilized included blood samples, Turk's solution for the leukocytes test, and Hayem's solution for the erythrocytes test. The instruments utilized included a Thoma leukocyte and erythrocyte pipette, a hemocytometer, and a microscope with a 10× magnification. The Sahli method was used to determine hemoglobin levels [15]. Blood samples, 0.1 N HCL, and distilled water were utilized. The tools employed were a Sahli pipette and a Hb-meter tube. Hematocrit was measured according to the protocol [16]. Blood samples and critoseal were used as materials, and microhematocrit and centrifugation were employed as equipment.

The distal intestine fixation was carried out using Neutral Bouin Formalin 30% for observation of histology samples. The preparations of histological slides were according to [17]. Histological preparation was conducted using xylol, touluol, eosin, hematoxylin, paraffin, and graded alcohol at 50%–96%**.** The histological results observation was performed with a microscope with 4 times magnification.

The proximate test consists of protein, carbohydrate, fat, and ash content measurements. The Kjeldahl method determined crude protein (ISO 5983, 1979). Fat measured by petroleum-diethyl ether extraction (ISO 6492, 1999), ash content determined by the gravimetric method (SNI 2354.1:2010), dan carbohydrates test by a different process (SNI 01-2370-1991). The proximate test used distilled water, HCl 0.1 N, alcohol 70%, H_2SO_4, K_2SO_4, NaOH, hydrochloric acid, stoichiometry, digital caliper, analytical balance digital, desiccator, Beaker Glass, petri disc, atomic absorption spectrophotometer, steam distillation, oven, and the Kjeldahl destruction tool.

2.3. Data analysis

Distal intestinal histology measurements were performed with the ImageJ program. Calculation of absolute and daily growth uses the formula of [18]. Furthermore, the FCR and SR values are obtained using the formula of [19], [20]:

$$\text{Relative growth rate (\%)} = \frac{\text{final weight or length} - \text{initial weight or length}}{\text{initial weight or length}} \times 100$$

$$\text{Specific growth rate (\%/day)} = \frac{\log(\text{final weight or length}) - \log(\text{initial weight or length})}{\text{time}} \times 100$$

$$\text{Feed conversion rate} = \frac{\text{the total amount of feed consumed}}{\text{final body weight} - \text{initial body weight} + \text{total body weight of dead fish}}$$

$$\text{Survival rate (\%)} = \frac{\text{Number of fish harvested}}{\text{Number of fish stocked}} \times 100$$

2.4. Statistical analysis

Growth data, digestive enzyme values, hematology, and histology were analyzed using statistical analysis in the form of a one-way Analysis of Variance (ANOVA) with a significance level of 0.05 and additional tests using the Duncan Multiple Range Test. Data processing was conducted using the IBM SPSS version 23.0 program.

3. Results

3.1. Fish Performance

Table 1 displays the outcomes of the dry BSF proximate test. The nutritional value consisted of 36.9%–37.4% protein, 32.7%–39.4% fat, 5.5%–8.5% carbohydrates, and 11.7%–15.6% ash.

The fish performance results during the study are presented in Table 2. It shows that the highest weight growth was in treatment B20, 509.17 ±_39.48%, without significant difference ($p \geq 0.05$). Additionally, treatment B20 showed the lowest value in body length growth, significantly different from the others, namely 75.67 ±_4.04% ($p \leq 0.05$). The SGR growth rate also indicated the highest value, significantly different from the others, namely 7.90 ± 0.48% ($p \leq 0.05$). The treatment B20 also showed a significantly different value, except for the B80, which showed no difference ($p \geq 0.05$). Fish survival during the B50 treatment study indicated the highest value of 80 ± 4.3%, but it was not significantly different ($p \geq 0.05$). The best FCR value occurred in treatment B80, namely 0.58 ±_0.06, but not significantly different from B20 (0.68_± 0.06) and B50 (0.69 ± 0.12) ($p \geq 0.05$). However, it was significantly different from the FCR of B100 (1.05_± 0.12) ($p \leq 0.05$).

3.2. Digestive enzymes

The results of the amylase, lipase, and protease enzyme tests are shown in Table 3. The P4 treatment had the highest mean value compared to others with amylase enzyme values (6.09 ±_0.24 IU/mL), lipase (0.44 ± 0.030 IU/mL), and protease (0.49_± 0.029 IU/ mL), which were significantly different ($p \leq 0.05$).

3.3. Meat content

The proximate test from dry matter of meat content in Table 4 showed that fat values did not significantly differ between treatments ($p \geq 0.05$). Concurrently, the value of fish carbohydrates in treatment B20 (8.69_± 0.47 %) had the lowest value, significantly different from the others ($p \leq 0.05$). The highest protein content was found in B100 (22.70 ± 0.04%), significantly different from B20 (21.73 ± 0.38%) ($p \leq 0.05$) but not B50 (22.47 ± 0.28%) and B80 (22.15_± 0.17 %) ($p \geq 0.05$).

3.4. Hematology (hematocrit, hemoglobin, leukocytes, and erythrocytes)

The haematological test in Table 5 showed that the total erythrocyte, haematocrit, and haemoglobin values were not significantly different between treatments, except for the leukocytes. The highest leukocyte value was found in B80 ($5.29 \pm 1.08 \times 10^4$ / mm^3) and was significantly different from the others ($p \leq 0.05$).

3.5. Distal intestinal histology

The morphology of the distal intestine is presented in Figure 1. The distal parts of the intestine consist of the mucosal fold height, width, and muscularis thickness. The pictures were taken using a microscope with 4X magnification.

The average value of the distal intestinal morphology is presented in Table 6. It shows that the highest mucosal fold height value was found in treatment B80 of 98.81 ± 7.30 µm and was not significantly different from B100 at 95.20_± 4.18 µm ($p \geq 0.05$). However, it differed significantly from the other two treatments. The highest mucosal fold width value also occurred in B80 (13.97 ± 0.78 µm) but was not significantly different from the others. Moreover, B80 also had the highest muscularis thickness value of 20.23_± 1.33 µm, significantly different from the other treatments ($p \leq 0.05$).

3.6. Water quality

The highest temperature, DO, pH , and average TDS were in treatment B20 (30.15 ± 1.82 °C), B100 (7.19_± 0.65 mg/L), B80 (7.75_± 0.64), and B80 (97.66_± 15.86 mg/L), respectively.

Table 1. The protein, fat, carbohydrate, and ash content of dry Black Soldier Fly (BSF) nutritional composition and commercial diet.

Nutrient content	BSF
Protein (%)	36.9–37.46
Fat (%)	32.77–39.4
Carbohydrate (%)	5.5–8.5
Ash content (%)	11.7–15.6
*BSF: Black Soldier Fly	

Table 2. Growth performance of cork fish in all treatments.

T	Initial weight (g) ± SE	Initial length (cm) ± SE	Final weight (g) ± SE	Final length (cm) ± SE	Relative growth rate		Specific growth rate		SR (%) ± SE	FCR ± SE
					Weight (%) ± SE	L (%) ± SE	Weight (%) ± SE	L (%) ± SE		
B20	1.65 ± 0.09^{a}	5.54 ± 0.10^{b}	9.6 ± 0.2^{a}	8.76 ± 0.44^{b}	509.17 ± 39.48^{a}	75.67 ± 4.04^{a}	7.90 ± 0.48^{a}	4.54 ± 0.22^{a}	70 ± 6.52^{a}	0.68 ± 0.06^{ab}
B50	1.55 ± 0.11^{a}	5.14 ± 0.11^{a}	10.14 ± 0.12^{b}	7.71 ± 0.24^{a}	502.84 ± 40.04^{a}	101.78 ± 4.58^{b}	6.83 ± 0.27^{b}	5.55 ± 0.18^{c}	80 ± 4.3^{a}	0.69 ± 0.12^{ab}
B80	2 ± 0.15^{b}	4.84 ± 0.07^{a}	9.24 ± 0.11^{a}	9.05 ± 0.33^{b}	455.64 ± 36.69^{a}	93.24 ± 3.63^{b}	7.80 ± 0.38^{ab}	4.88 ± 0.18^{ab}	65 ± 7.51^{a}	0.58 ± 0.06^{a}
B100	1.43 ± 0.09^{a}	4.83 ± 0.05^{a}	9.67 ± 0.12^{a}	7.09 ± 0.23^{a}	482.77 ± 35.73^{a}	102.92 ± 4.29^{b}	6.25 ± 0.30^{b}	5.37 ± 0.17^{*bc}	78 ± 4.4^{a}	1.05 ± 0.12^{b}

*B20: group received 20% Black Soldier Fly feed, B50: group received 50% Black Soldier Fly feed, B80: group received 80% Black Soldier Fly feed, B100: group received 100% Black Soldier Fly feed.

**T: treatment, L: total length, SE: standard error, SR: survival rate, FCR: feed conversion ratio

Mean values with different superscripts in the same column are significantly different at $p < 0.05$.

Table 3. During the study, the average value of cork fish's amylase, lipase, and protease.

Treatment	Amylase ± SE (IU/mL)	Lipase ± SE (IU/mL)	Protease ± SE (IU/mL)
B20	3.62 ± 0.03[a]	0.15 ± 0.01[a]	0.26 ± 0.00[a]
B50	5.41 ± 0.12[b]	0.34 ± 0.01[b]	0.39 ± 0.00[b]
B80	4.36 ± 0.12[c]	0.21 ± 0.00[c]	0.30 ± 0.00[c]
B100	6.09 ± 0.08[d]	0.44 ± 0.01[d]	0.49 ± 0.01[d]

*B20: 20% Black Soldier Fly feed was provided to the group, B50: 50% Black Soldier Fly feed was provided to the group, B80: 80% Black Soldier Fly feed was provided to the group, B100: 100% Black Soldier Fly feed was provided to the group.Mean values with different superscripts in the same column are significantly different at $p < 0.05$.

Table 4. Value of fat, carbohydrate, and protein content of cork fish meat in each treatment.

Treatment	Fat ± SE (%)	Carbohydrate ± SE (%)	Protein ± SE (%)
B20	8.69 ± 0.47[a]	1.04 ± 0.15[a]	21.73 ± 0.38[a]
B50	9.74 ± 0.60[a]	2.01 ± 0.19[b]	22.47 ± 0.28[ab]
B80	9.4 ± 0.33[a]	1.76 ± 0.09[b]	22.15 ± 0.17[ab]
B100	10 ± 0.50[a]	2.15 ± 0.12[b]	22.70 ± 0.04[b]

*B20: group received 20% Black Soldier Fly feed, B50: group received 50% Black Soldier Fly feed, B80: group received 80% Black Soldier Fly feed, B100: group received 100% Black Soldier Fly feed.
Mean values with different superscripts in the same column are significantly different at $p < 0.05$.

Table 5. Total values of cork fish's leukocytes, erythrocytes, hematocrit, and hemoglobin.

Treatment	Total Leukocytes ± SE ($\times 10^4$/mm^3)	Total Erythrocytes ± SE ($\times 10^6$/mm^3)	Hematocrit ± SE (%)	Hemoglobin ± SE (g/100 mL)
B20	1.75 ± 0.18[a]	1.45 ± 0.09[a]	54.7 ± 3.33[a]	9.35 ± 0.25[a]
B50	2.53 ± 0.80[a]	1.30 ± 0.08[a]	53.12 ± 3.72[a]	8.75 ± 0.36[a]
B80	5.29 ± 1.08[b]	2.38 ± 0.80[a]	50.89 ± 3.9[a]	9.75 ± 0.41[a]
B100	1.98 ± 0.18[a]	3.22 ± 1.37[a]	54.01 ± 1.55[a]	9.31 ± 0.31[a]

*B20: 20% Black Soldier Fly feed was provided to the group, B50: 50% Black Soldier Fly feed was provided to the group, B80: 80% Black Soldier Fly feed was provided to the group, B100: 100% Black Soldier Fly feed was provided to the group.
Mean values with different superscripts in the same column are significantly different at $p < 0.05$.

4. Discussion

The dose of BSF mixed feed is inversely proportional to the increase in body weight and specific growth rate. The same result was obtained in pearl gentian grouper fish [21] and rainbow trout [22] fed a BSF mixture. It was explained that carnivorous fish require nutrients from lysine and arginine, which are lower in BSF [21]. Therefore, it does not positively impact growth even though the BSF dose is increased.

The highest growth in fish weight was in treatment B20, which was fatter than the others. This is indicated by the value of the average specific growth rate (SGR) showing the best results with a good FCR. In previous studies, feeding FM to snakehead fish resulted in lower SGR values, i.e. 1.16% and 6.19%/day [23], [24]. The results are similar to *Seriola quinqueradiata*, *Pelteobagrus fulvidraco*, *Acipenser baeri*, and *Oncorhynchus mykiss* [8], [10], [25], [26], which showed negative growth in the tested fish when the BSF substitution was ≥20%. This is also supported by Prokoso et al. [11], where using substitutes at levels ≥30% reduced growth in carnivorous fish. Meanwhile, the substitution of less than 20% in *Clarias gariepinus*, *Cyprinus carpio*, and

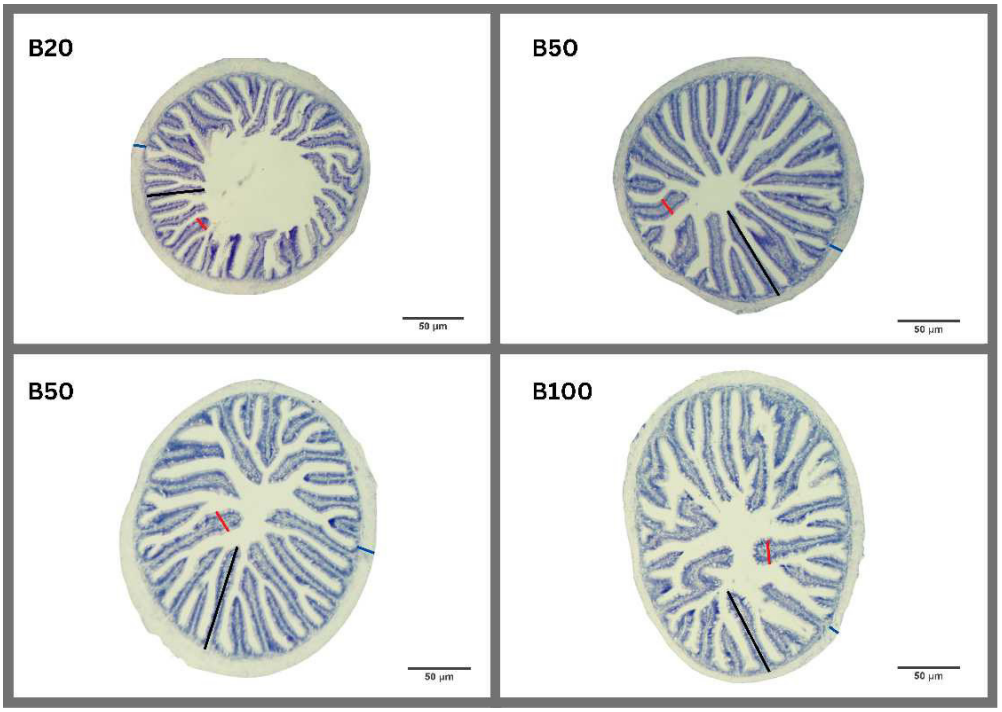

Figure 1. Distal intestinal morphology. Black color represents mucosa fold height, red indicates muscosal fold width, and blue represents muscularis thickness.
B20: group received 20% Black Soldier Fly feed, B50: group received 50% Black Soldier Fly feed, B80: group received 80% Black Soldier Fly feed, B100: group received 100% Black Soldier Fly feed.

Table 6. Distal intestinal morphological average values include mucosal fold height, width, and muscularis thickness.

Treatment	Mucosal fold height (µm ± SE)	Mucosal fold width (µm ± SE)	Muscularis thickness (µm ± SE)
B20	76.10 ± 2.46^{a}	12.29 ± 0.50^{a}	11.33 ± 0.47^{a}
B50	80.01 ± 2.59^{ab}	13.50 ± 0.62^{a}	13.49 ± 0.68^{a}
B80	98.81 ± 7.30^{c}	13.97 ± 0.78^{a}	20.23 ± 1.33^{b}
B100	95.20 ± 4.18^{bc}	12.06 ± 0.70^{a}	12.13 ± 0.61^{a}

*B20: group received 20% Black Soldier Fly feed, B50: group received 50% Black Soldier Fly feed, B80: group received 80% Black Soldier Fly feed, B100: group received 100% Black Soldier Fly feed.
Mean values with different superscripts in the same column are significantly different at $p < 0.05$.

Monopteros albus resulted in a positive impact on growth [27]–[29]. In contrast, a study by on Nile tilapia showed negative growth results. This is presumably due to differences in the sources of BSF food ingredients which can affect the nutritional value quality [30]. In contrast, giving a BSF of no more than 20% shows good average growth in this study. Similarly, adding up to 19.5% BSF to the diet of European seabass had no negative effects on growth performance or digestive enzyme activity [31]. The percentage of BSF for substituted fish meals has varying growth effects for each fish species, which could be influenced by the fish size and species, insect species, insect cultivation, and insect processing [22].

The nutritional value of fish consumption consists of 25%–55% protein, 10%–40% carbohydrates, and 2%–50% fat [32]. The nutritional components of dry BSF can contain protein and fat up to 50% and 35%, respectively [33]. Meanwhile, the total protein value in this study was lower, while the fat content in BSF was higher than the commercial diet. In addition to environmental factors, such findings could be caused by the feed consumed by the BSF and the drying process [34]. High-fat content in feed can cause growth disturbances and impact low fish survival [11]. It was proven that all treatments had a low average SR value, and the addition of BSF feed composition can affect the

increase in excess intestinal microbes while decreasing fish health [21].

The intestine is the organ to observe the digestion and absorption of feed in fish [10]. Good intestinal digestibility performance can be evaluated from histological results [35]. Based on intestinal histological analysis, the addition of BSF as a substitute feed for FM is reported to have a negative indication because it causes the mucosal folds and muscular to shrink [21], [29]. This condition can affect low intestinal performance and cause damage [29]. The mucosal folds affect the intestinal surface's absorption ability, and the muscular determines the peristaltic and intestinal motility [36]. Subsequently, a normal mucosa will affect the function of digestion and good absorption in the intestine [36]. This slowed the development of the fish, especially those fed a 20% BSF diet. This may be due to the fact that BSF has a low concentration of polyunsaturated fatty acids, which can reduce the efficiency of intestinal metabolism [21] and these nutrients are affected by the maintenance and processing of BSF [22].

Fish digestive enzymes assist the digestion and absorption of feed nutrients by the fish intestine into simpler nutrients. Fish nutritional intakes, such as protein, carbohydrates, and fat, have an impact on mechanisms of digestive enzymes [32], [37]. Amino acid as building blocks of protein intake influences the synthesis and activity of protease enzymes in protein digestion, as well as the metabolism of amino acids. Consuming carbohydrates has an impact on the production and function of amylase enzymes in glucose metabolism and carbohydrate digestion. Ingestion of fat affects the synthesis and activity of lipase enzymes in fat digestion, as well as fatty acid metabolism. In this study, increasing the BSF doses showed an increase in the value of lipase, amylase, and protease enzymes but did not significantly have an effect on the growth. A similar result was obtained in previous studies conducted on grouper fish [21]. This is presumably due to the high BSF lipid content, which can suppress fish growth, as seen in the lemon fin barb hybrid [38]. Fat can affect the fish's digestive performance [11], hence it needs to be reduced for proper digestion. Another cause is the high chitin content in BSF, which adversely affect nutrient digestibility [7], [39]. Furthermore, it is also the main component in insect bodies [40]. Fish fed with BSF show chitin degradation in their stomachs and intestines [41] due to the chitinase enzyme [7]. Intestinal microflora also catalyzes chitin [7], but both are still being debated. This study did not observe chitinase enzymes and intestinal microflora. However, it had a good role in the test fish because the investigation of the intestinal mucosa showed good digestibility.

The proximate test showed that the fat value of fish meat was not significantly different between treatments. However, there was an increase in carbohydrates and protein with increasing BSF doses in the feed. Previous research showed the composition of protein and fat in snakehead fish meat given FM was lower than this study, i.e. 17.08% and 3.08%, respectively [42]. This implies that giving BSF up to 100% can increase the protein content. A similar study also occurred in crock fish farming fed with BSF, showing a higher protein content than FM [43]. It is suspected by the performance of the chitinase enzyme and the metabolic flora in the intestines of cork fish which work well to digest BSF. The chitin content negatively affects the digestive system and protein [7]. Therefore, the correct dosage must be considered when using BSF as a substitute for fish nutrition, growth, and reproduction [44] for further studies.

Hematologic parameters are used to investigate the fish's health status and stress level due to physiological conditions to changes in environment and feed [9]. Prior research demonstrated that the values of hematocrit, erythrocytes, and leukocytes in snakehead fish given FM were lower than those observed in the present study, i.e. 24.4%, 2.5×10^6 /mm^3, and 1.53×10^4/mm^3, respectively [45]. The leukocyte value increased with the BSF dose, especially in treatment B80, and was significantly different from the others. However, this value is still under normal conditions compared to cork fish farming, which FM by 62.9×10^3 cells/mm^3 [46]. Erythrocyte values between treatments were not significantly different but increased with high doses of BSF. The value is above the normal standard at $1.05–3.00 \times 10^6$ cell/mm^3 [47]. This is due to the rearing of fish outdoors which causes the water temperature to increase. Furthermore, the water temperature can increase the erythrocytes [48] as a mechanism for fish to reduce stress [49]. It also occurs in salmon with high erythrocytes due to increased water temperature [50]. Additionally, treatment by increasing the BSF dose is likely to improve the content of unsaturated fatty acids, which are oxidized to free radicals [51] and consequently affect the number of erythrocytes. The haematocrit and haemoglobin values of the fish were in the normal range, namely 33%–55% and 8.1%–11% [47]. Based on their haematology, the fish's health is in good condition in all treatments.

The observed water quality, such as temperature, DO, pH and TDS, showed no significant differences between treatments. However, the temperature used was higher than in a study conducted by [47]. The increase in temperature can affect the fish's health and total erythrocytes. The temperature range was under normal conditions for fish rearing, between 26.8–32.5 °C. Meanwhile, the water quality was in good shape to support cork farming.

In conclusion, fresh meal B20 significantly affects daily weight growth, and B100 impacts increasing levels of digestive enzymes and meat content (protein and carbohydrates) compared to other BSF groups. In general, the

administration of BSF in snakehead fish farming in this study was tolerated by intestinal digestion and had a haematological value within normal limits. However, further research is needed to compare BSF with other fish meals in Indonesia with various nutrient compositions.

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Informed consent
This study protocol has been evaluated by the Ethical Clearance Commission of Universitas Gadjah Mada. The code for this certificate of ethics is 00135/04/LPPT/III/2019.

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