Research Article

Influences of Partial Substitution of Fish Meal with Defatted Black Soldier Fly (*Hermetia illucens*) Larvae Meal in Diets on Growth Performance, Biochemical Parameters, and Body Composition of Juvenile Chinese Soft-Shelled Turtles (*Pelodiscus sinensis*)

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The black soldier fly (*Hermetia illucens*) larvae meal (BSFM) has been widely applied in aquaculture production due to its rich nutritional value, easy availability, and renewability. However, to our knowledge, it has not been used in the diets for turtles. Here, we evaluated the acceptability of partial substitution of fish meal (FM) with defatted BSFM in the diets of juvenile Chinese soft-shelled turtles (*Pelodiscus sinensis*). A total of 225 juvenile turtles were randomly divided into D1, D2, D3, D4, and D5 groups, which were fed with BSFM replacing 0, 5%, 10%, 15%, and 20% FM, respectively, for 70 days. The results showed that growth performances of groups D2 and D3 were not significantly different ($P > 0.05$) from that of group D1, whereas the growth performance compromised ($P < 0.05$) in groups D4 and D5. The activity of serum alkaline phosphatase was higher ($P < 0.05$) in group D3 relative to other groups, while alanine aminotransferase and aspartate aminotransferase activities were higher ($P < 0.05$) in group D4 when compared with groups D1, D2, and D3. Liver lysozyme activity and malonaldehyde level were significantly higher ($P < 0.05$) in group D1 relative to other groups, whereas total antioxidant capacity activity showed the opposite trend ($P < 0.05$). The activities of hepatic total superoxide dismutase and glutathione peroxidase displayed a linear elevation ($P = 0.011$ and $P < 0.001$, respectively) from groups D1 to D3. Intestinal amylase and protease activities linearly increased ($P < 0.001$) with increasing BSFM levels, while intestinal lipase activity showed a quadratic increase and then a decrease with increasing BSFM levels ($P = 0.011$). Muscle crude protein and fat contents increased ($P < 0.05$) in group D3 when compared with groups D1 and D2. Muscle phenylalanine, glutamic, tryptophan, and arginine levels increased in group D1 in comparison with the other groups ($P < 0.05$), whereas the opposite was true for isoleucine and proline levels. The broken-line analysis based on specific growth rate estimated that the optimal level for replacing dietary FM with BSFM is 5.0%, which could elicit benefits on both the growth performance and physiological health condition of the juvenile Chinese soft-shelled turtles.

1. Introduction

Aquatic animals have become an important protein source because of the improvement in human living standards. This has greatly promoted the rapid development of the aquaculture industry; however, reduction in fish meal (FM) production seriously restricts the sustainable and healthy development of aquaculture. Therefore, renewable resources that can replace FM need to be urgently found. Recently, insect meals (black soldier fly, mealworm, and grasshopper) have received great attention as new raw materials because insects are easy to raise and grow fast and they have a wide range of food sources (e.g., livestock, poultry, and food waste), high feed conversion efficiency, and low environmental impact [1, 2]. Notably, production from insect farming is approximately 500 tons per year, which is an upscaling branch of animal production; the annual output of the European Union alone will reach 250,000 tons by 2030 [3]. Therefore, there will be a gradual reduction of the cost of insect meals along with an increase in their availability. These can support the possibility of using insect meals in animal diets. In fact, the European Union has authorized the application of insect meals in aquaculture production (Regulation 2017/893/EC, 2017), which greatly promotes the applications of insect-related products.

Black soldier fly (*Hermetia illucens*) larvae meal (BSFM), a highly nutritional meal, has been evidenced to have high levels of crude protein and crude fat along with well-balanced amino acid (AA) profiles comparable to those of FM [4, 5]. For fish and shrimp culture, BSFM has been evaluated as a replacement for dietary FM. A previous study has shown that when BSFM levels in the diet were lower than 15.78%, intestinal microbiota composition of rice field eel (*Monopterus albus*) improved; however, excessive levels of BSFM adversely affected the lipid metabolism [6]. In Nile tilapia juveniles (*Oreochromis niloticus*), the absolute substitution of dietary FM with BSFM failed to weaken the growth performance or innate immune responses [7]. Recent studies have indicated that supplemental BSFM at low or moderate (5–30%) levels could improve or not compromise the growth performance and physiological health condition in carnivorous fishes such as Siberian sturgeon (*Acipenser baerii*) [8], Japanese seabass (*Lateolabrax japonicus*) [9], Atlantic salmon (*Salmo salar*) [10], rainbow trout (*Oncorhynchus mykiss*) [11], and European seabass (*Dicentrarchus labrax*) [12]. In addition, the level of dietary FM substituted by BSFM may be limited to 20% or less for the Pacific white shrimp (*Litopenaeus vannamei*) [13]. Nevertheless, Wang et al. [14] found that BSFM could replace up to 60% of FM in diet without impairing growth performance and physiological health in Pacific white shrimp. These studies suggested a feasibility of BSFM replacing FM in diets of various fish and shrimp. However, the physiologies of fish and shrimp are quite different from that of turtles, and so far, little information was available regarding the replacement of dietary FM with BSFM for turtles.

The Chinese soft-shelled turtle (*Pelodiscus sinensis*) is broadly spread in certain Asian countries (e.g., China, Japan, Vietnam, and South Korea); it is carnivorous and mainly

feeds on fish, shrimp, and molluscs [15]. The production of Chinese soft-shelled turtle reached ~322,100 tons in 2017 because it is nutritious and delicious [16]. Growing evidences suggested that the requirements of juvenile turtles for lipid, protein, and starch are 6.38%, 42.20%, and 12.73%, respectively [17–19]. In terms of the Chinese soft-shelled turtle, substitution of dietary FM with plant protein ingredients including fermented soybean, soy protein concentrates, and rice protein concentrate has yielded positive results [20–22]. However, to date, there is no information regarding the application of BSFM in turtle diets, and it is known that the Chinese soft-shelled turtle has a habit of eating insects. Therefore, the present study was designed to investigate the influences of partial replacement of FM with defatted BSFM in diets on growth performance, biochemical parameters, and body composition of juvenile Chinese soft-shelled turtles.

2. Materials and Methods

2.1. Defatted BSFM. Defatted BSFM was bought from Shandong Wooneng Agricultural Science and Technology Co., Ltd. (Liaocheng, China). The nutritional ingredients of BSFM included 92.88% dry matter (DM), 46.09% crude protein, 11.37% crude fat, and 8.51% ash.

2.2. Experimental Design and Diets. All studies were performed under the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH Publications No. 8023, revised 1978) and approved by Liaocheng University (Shandong, China). A total of 225 juvenile Chinese soft-shelled turtles were purchased from a local farm and then raised in a pool (2.5 m × 2.5 m × 1.0 m) in the BSF breeding base of Liaocheng University. The turtles received a commercial diet for two weeks to acclimate to the new environment. Afterwards, healthy turtles with similar initial body weight (IBW; 39.90 ± 0.31 g) were stochastically divided into 15 tanks (1.0 m × 1.5 m), with three replicates per group and 15 turtles in each replicate. The water temperature was kept at 28–30°C, the pH of water was maintained at 7.2–7.4, the dissolved oxygen concentration was not lower than 5.0 µg/mL, and the ammoniacal nitrogen concentration was less than 0.5 µg/mL.

The basal FM diet served as the control diet (D1) without defatted BSFM. Diets D2, D3, D4, and D5 were prepared by supplementing 5%, 10%, 15%, and 20% defatted BSFM, respectively, into diet D1 at the expense of FM. The five diets were prepared based on isoproteic (about 48.1%) and isolipidic (about 7.0%) design. The crude protein contents in FM, soybean meal, and corn gluten meal were 62.48%, 45.96%, and 60.67%, respectively, while the crude fat contents in these ingredients were 6.19%, 1.73%, and 4.85%, respectively. All ingredients were smashed via a 60-mesh sieve and underwent thorough blending step by step, followed by sufficient mixing with oil. The prepared diets were reserved at -20°C. Before feeding the turtles, 25% water was added to the diets, and slivers were made using a small hand noodle machine (MC-2; Yongkang Yongwei Machinery Factory, Zhejiang, China). The nutritional composition of the diets is listed in Table 1, and dietary amino acid and fatty acid patterns are shown in Table 2.

TABLE 1: The nutritional composition of the experimental diets (air-dry basis (g/kg)).

Item	D1	D2	Group D3	D4	D5
	Ingredient				
Fish meal	600.0	570.0	540.0	510.0	480.0
Black soldier fly larvae meal ^a	0.0	41.5	83.0	124.5	166.0
Chicken meal	20.0	20.0	20.0	20.0	20.0
Corn gluten meal	60.0	60.0	60.0	60.0	60.0
Soybean meal	60.0	60.0	60.0	60.0	60.0
Soybean oil	12.0	10.2	8.4	6.6	4.8
α -Starch	218.0	208.3	198.6	188.9	179.2
Ca(H ₂ PO ₄) ₂	20.0	20.0	20.0	20.0	20.0
Premix ^b	10.0	10.0	10.0	10.0	10.0
Total	1000.0	1000.0	1000.0	1000.0	1000.0
	Proximate analysis ^c				
Moisture (%)	15.13	15.00	14.76	15.92	14.88
Energy (MJ/kg)	19.24	19.27	19.29	19.32	19.33
Crude protein (%)	48.14	48.13	48.19	48.10	48.04
Crude lipid (%)	6.96	7.00	7.04	7.09	7.13
Ash (%)	16.92	16.61	16.30	15.98	15.67

^aProvided by Shandong Woneng Agricultural Technology Co., Ltd. (Shandong, China). ^bObtained from Turtle Farm of Liaocheng Economic and Technological Development Zone (Shandong, China). ^cBased on the sample analysis in triplicate.

2.3. Sampling Procedures. At the end (d 70) of the experiment, the final body weight (FBW) of turtles was determined after starvation for 24 h. Thereafter, 12 turtles were stochastically chosen from each group (4 turtles per replicate) and anesthetized using 0.01% 2-phenoxyethanol (Sigma-Aldrich, St. Louis, USA). Subsequently, blood was taken from the tail vein, followed by centrifugation (5,000 $\times g$, 4°C, 10 min) to obtain the serum samples, which were then reserved at -80°C for biochemical analysis. After collection of blood, the turtles were sacrificed, and the liver, small intestine, and leg muscle were separated and quick-frozen by liquid nitrogen, followed by storage at -80°C for further analysis.

2.4. Determination of Growth Performance. The growth performances including weight gain rate (WGR), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR) of the turtles were calculated as follows:

$$\text{WGR (\%)} = 100 \times \left(\frac{\text{FBW} - \text{IBW}}{\text{IBW}} \right), \quad (1)$$

$$\text{SGR (\%)} = 100 \times \left(\frac{\text{Ln (FBW)} - \text{Ln (IBW)}}{\text{days}} \right), \quad (2)$$

$$\text{FCR} = \frac{\text{diet consumed (g)}}{\text{weight gain (g)}}, \quad (3)$$

$$\text{SR (\%)} = 100 \times \left(\frac{\text{final number of fish}}{\text{initial number of fish}} \right). \quad (4)$$

TABLE 2: Amino acid and fatty acid compositions of the experimental diets (% dry matter basis).

Item	D1	D2	D3	D4	D5
	Amino acids ^a				
Lys	3.48	3.45	3.41	3.35	3.31
Phe	2.11	2.18	2.24	2.31	2.38
Met	1.17	1.17	1.18	1.19	1.19
Thr	2.04	2.00	1.98	1.95	1.93
Ile	2.05	2.04	2.02	2.01	1.99
Leu	4.04	3.98	3.95	3.90	3.86
Val	2.35	2.26	2.16	2.07	1.98
Try	0.56	0.63	0.71	0.78	0.85
His	1.34	1.33	1.31	1.30	1.28
Arg	2.88	2.89	2.89	2.89	2.89
Cys	0.51	0.52	0.53	0.52	0.53
	Fatty acids ^b				
C12:0	0.00	0.42	0.83	1.25	1.66
C14:0	3.78	3.72	3.68	3.63	3.58
C16:0	12.34	12.61	12.87	13.14	13.4
C18:0	2.91	2.91	2.91	2.91	2.91
SFA	19.03	19.66	20.29	20.93	21.55
C16:1	4.99	4.90	4.82	4.73	4.65
C18:1	8.16	9.01	9.87	10.73	11.58
MUFA	13.15	13.92	14.69	15.46	16.23
C18:2n-6	8.55	9.02	9.49	9.97	10.44
C20:4n-6	1.48	1.41	1.35	1.28	1.22
n-6 PUFA	10.03	10.43	10.84	11.25	11.66
C18:3n-3	0.95	0.98	1.01	1.04	1.07
C20:5n-3	3.75	3.56	3.38	3.19	3.01
C22:5n-3	2.03	1.93	1.83	1.72	1.62
C22:6n-3	8.98	8.53	8.08	7.63	7.19
n-3 PUFA	15.71	15.00	14.30	13.58	12.89

^aLys: lysine; Phe: phenylalanine; Met: methionine; Thr: threonine; Ile: isoleucine; Leu: leucine; Val: valine; Asp: aspartic acid; Ser: serine; Glu: glutamic acid; Gly: glycine; Ala: alanine; Try: tryptophan; His: histidine; Arg: arginine; Pro: proline. ^bSFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

2.5. Assay of Serum Biochemical Indices and Enzyme Activity in the Intestine. Serum samples were gained from blood as described above. The intestine samples were homogenized in 0.90% saline solution (1:9 *m/v*) and then centrifuged at 2,000 rpm/min for 15 min at 4°C for supernatant collection. The concentrations of total protein (TP), globulin (GLOB), albumin (ALB), cholesterol (CHOL), and triglycerides (TG) together with the activities of serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lysozyme (LZM) and the activities of intestinal amylase, lipase, and protease were measured with corresponding commercial kits (Jiancheng Bioengineering Institute, Nanjing, China; catalog nos. A045-4-2, A028-2-1, A111-1-1, F001-1-1, A059-2-2, A050-1-1, C009-2-1, C010-2-1, C016-1-1, A080-1-1, and A054-2-1 for the analysis of TP, ALB, CHOL, TG, ALP, LZM, ALT, AST, amylase, protease, and lipase, respectively). All

indicators were measured following the manufacturer's instructions.

2.6. Measurements of Hepatic Antioxidant and Oxidation Parameters. The liver sample was homogenized in 0.90% saline solution (1:9 *m/v*) and then centrifuged at 2,000 rpm/min for 15 min at 4°C; the resulting supernatants were then collected. The activities of hepatic LZM, total antioxidant capacity (T-AOC), catalase (CAT), glutathione peroxidase (GSH-Px), and total superoxide dismutase (T-SOD) coupled with malondialdehyde (MDA) content were measured with commercial kits (Jiancheng Bioengineering Institute, Nanjing, China; catalog nos. A050-1-1, A015-3-1, A005-1-2, A001-1-2, A007-1-2, and A003-1-2 for the analysis of LZM, T-AOC, GSH-Px, T-SOD, CAT, and MDA, respectively). All indicators were measured under the manufacturer's protocols.

2.7. Proximate Compositions and AA Profile Assay. The proximate compositions of diets and muscle were analyzed according to the study of Li et al. (2017) [23]. The moisture content of the samples was determined by high-temperature drying (105°C) for 2 h. The crude protein was quantified by Kjeldahl method after acid treatment. The crude fat was quantified using the Soxhlet extractor and petroleum ether at 30–60°C for 1 h, while the content of ash was measured by carbonization in a battery oven, followed by ashing at 550°C for 2 h in a muffle furnace.

The AA profiles of the muscles were measured according to a previous study (Hamzeh et al., 2015 [24]). The muscle samples were firstly treated by 6 mol/L HCl at 110°C under N₂ for 22 h and then analyzed using the Amino Acid Analyzer (Hitachi L-8900; Hitachi, Ltd., Tokyo, Japan). The analyzed AAs included both essential amino acids (EAAs) (e.g., methionine (Met), lysine (Lys), phenylalanine (Phe), threonine (Thr), leucine (Leu), isoleucine (Ile), and valine (Val)) and nonessential amino acids (NEAAs) (e.g., alanine (Ala), glycine (Gly), serine (Ser), tryptophan (Try), glutamic acid (Glu), aspartic acid (Asp), arginine (Arg), histidine (His), and proline (Pro)).

2.8. Analysis of Fatty Acid Compositions. The fatty acid profiles of the diet were analyzed in accordance with the report of Li et al. [25]. The lipid in the samples was firstly extracted using mixed solvents (chloroform/methanol, 2:1 *v/v*) and converted to fatty acid methyl esters (FAMES) using 0.5 mol/L KOH-methanol and boron trifluoride etherate. FAMES were detected by means of a gas chromatograph (7890B; Agilent Technologies, Palo Alto, USA) with a capillary column (60 m × 0.25 mm i.d., 0.20 μm film thickness; CP7487; Agilent). After sample injection (1 μL), the oven temperature of column was maintained at 140°C for 5 min, followed by increase from 140 to 220°C at 4°C/min and maintenance at 220°C for 10 min. The detector temperatures were maintained at 300°C. The fatty acids in the sample were finally identified through comparison with certain standards (CRM47885; Sigma-Aldrich, St. Louis, USA).

2.9. Statistical Analysis. The data were expressed as the mean ± standard error of the mean (SEM) and analyzed by one-way ANOVA in SPSS 25.0. Differences between groups

were detected by Tukey's test. In addition, orthogonal polynomial contrasts were employed to test the linear and quadratic effects of dietary defatted BSFM levels. Significant differences were set at $P < 0.05$.

3. Results

3.1. Growth Performance. As shown in Table 3, the IBW of the juvenile Chinese soft-shelled turtles was not significantly different ($P > 0.05$) among groups. Dietary defatted BSFM levels had no effects ($P > 0.05$) on the FBW and SR of the turtles. Groups D1 and D2 had higher ($P < 0.05$) WGR compared with group D5, with no significant differences ($P > 0.05$) observed between group D3 and other groups. Groups D1 and D3 had higher ($P < 0.05$) SGR relative to groups D4 and D5. The FCR significantly and linearly elevated ($P < 0.05$; $P < 0.001$) with increase of BSFM levels in groups D3 to D5. The broken-line analysis of SGR estimated the proper level of dietary defatted BSFM at 5.0% (Figure 1).

3.2. Digestive Enzyme Activity in the Intestine. A significant reduction ($P < 0.05$) of the amylase activity was observed in groups D1 and D2 relative to other groups (Figure 2). The intestinal protease activity showed a significantly linear increase from groups D1 to D4 ($P < 0.05$; $P < 0.001$); however, there was no significant difference ($P > 0.05$) in intestinal protease activity between groups D4 and D5. Groups D2 and D3 had significantly higher ($P < 0.05$) lipase activity versus group D5. Besides, intestinal lipase activity showed a quadratic increase and then a decrease with increasing BSFM levels ($P < 0.05$).

3.3. Biochemical Parameters in Serum. The defatted BSFM concentrations in diet had no effects ($P > 0.05$) on the concentrations of serum ALB, CHOL, and TG of the turtles (Table 4). There was a significantly higher ($P < 0.05$) concentration of TP in groups D1 and D2 compared with group D5, while group D2 had a significantly higher ($P < 0.05$) concentration of GLOB than other groups. Group D3 had a significantly higher ($P < 0.05$) activity of serum ALP relative to other groups. There was a significantly higher ($P < 0.05$) activity of LZM in group D1 than other groups; besides, LZM activity displayed a linear reduction ($P < 0.05$) in response to the increase of dietary BSFM levels. Group D4 had a significantly higher ($P < 0.05$) activities of serum ALT and AST than those in groups D1, D2, and D3, and the increased ALT activity showed a linear trend ($P < 0.05$).

3.4. Hepatic Antioxidant and Oxidation Parameters. As shown in Table 5, the dietary defatted BSFM levels had no effects ($P > 0.05$) on the activities of CAT in the liver of the turtles. Group D1 had significantly higher ($P < 0.05$) LZM activity and MDA level in the liver than other groups, whereas T-AOC activity displayed a converse trend ($P < 0.05$). Moreover, the LZM activity exhibited a linear reduction with the increase of BSFM levels ($P < 0.05$). With regard to hepatic GSH-Px activity, it was found to be significantly elevated ($P < 0.05$) with the supplementation of BSFM from groups D1 to D3, with the value peaking in the D3 group.

TABLE 3: Effects of different substitution levels of BSFM for FM in diets on growth performance in juvenile Chinese soft-shelled turtles.

Item	D1	D2	D3	D4	D5	P value	Linear	Quadratic
IBW (g)	39.9 ± 0.68	39.77 ± 0.76	39.80 ± 0.73	39.95 ± 0.67	40.07 ± 0.85	0.998	0.817	0.947
FBW (g)	78.21 ± 1.47	78.36 ± 1.08	77.57 ± 1.29	75.42 ± 1.69	75.64 ± 1.54	0.436	0.072	0.199
WGR (%)	95.99 ± 0.85 ^c	94.77 ± 1.51 ^{bc}	91.72 ± 2.90 ^{abc}	90.29 ± 1.56 ^{ab}	88.79 ± 0.93 ^a	0.015	0.000	0.002
SGR	1.20 ± 0.01 ^b	1.21 ± 0.02 ^b	1.19 ± 0.04 ^{ab}	1.13 ± 0.02 ^a	1.13 ± 0.01 ^a	0.019	0.002	0.008
FCR	2.25 ± 0.09 ^a	2.24 ± 0.08 ^a	2.48 ± 0.09 ^a	2.76 ± 0.06 ^b	3.00 ± 0.06 ^c	0.000	0.000	0.000
SR (%)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	—	—	—

Data are expressed as the mean ± SEM ($n = 3$). Values without a common superscript differ significantly ($P < 0.05$). IBW: initial body weight; FBW: final body weight; WGR: weight gain rate; SGR: specific growth rate; FCR: feed conversion ratio; SR: survival rate.

FIGURE 1: Broken-line model for the specific growth rate of juvenile Chinese soft-shelled turtles received diets with different substitution levels of BSFM for FM.

FIGURE 2: Effects of different substitution levels of BSFM for FM in diets on intestinal enzyme activities in juvenile Chinese soft-shelled turtles. Data are expressed as the mean ± SEM ($n = 6$). ^{a-d}Values without a common superscript differ significantly ($P < 0.05$). L: linear; Q: quadratic.

3.5. Proximate and AA Profiles of the Muscle. The dietary defatted BSFM levels had no effects ($P > 0.05$) on the moisture content of the muscle (Table 6). A significantly higher ($P < 0.05$) level of crude protein was observed in group D3 relative to groups D1 and D2. The content of crude fat in the muscle was linearly elevated ($P < 0.001$) with increasing

BSFM levels ($P < 0.05$). In terms of the ash content, it was found to be significantly increased ($P < 0.05$) in group D1 when compared with groups D4 and D5.

The AA profiles of the muscle are shown in Table 6; the dietary defatted BSFM levels had no effects ($P > 0.05$) on the levels of EAA, namely, Lys, Met, Thr, Leu, and Val, as well as the levels of NEAA, namely, Gly, Ala, Asp, and His in the muscle. The Phe level was found to be significantly higher ($P < 0.05$) in group D1 than other groups, whereas the opposite was true for Ile level ($P < 0.05$). Groups D3 and D4 had significantly lower ($P < 0.05$) level of Ser versus groups D2 and D5. Comparatively, both the Glu and Arg levels exhibited a linear reduction ($P \leq 0.001$ and $P < 0.001$, respectively) in response to the increase of BSFM levels. Furthermore, the Try level was significantly higher ($P < 0.05$) in group D1 relative to other groups, while the level of Pro was significantly decreased ($P < 0.05$) in groups D1 and D2 relative to groups D4 and D5.

4. Discussion

In the present study, we detected no differences in both FBW and SR among all groups, indicating a feasibility of the partial substitution of FM with BSFM in turtle diets. However, WGR and SGR values significantly decreased and FCR values increased in turtles received diets containing 15% and 20% BSFM as compared with those received FM-based

TABLE 4: Effects of different substitution levels of BSFM for FM in diets on serum biochemical parameters in juvenile Chinese soft-shelled turtles.

Item	D1	D2	D3	D4	D5	<i>P</i> value	Linear	Quadratic
TP (g/L)	38.60 ± 0.49 ^b	38.91 ± 0.51 ^b	37.35 ± 0.66 ^{ab}	37.42 ± 0.72 ^{ab}	36.24 ± 0.53 ^a	0.013	0.001	0.004
ALB (g/L)	16.94 ± 0.42	17.31 ± 0.45	16.96 ± 0.49	17.20 ± 0.46	16.75 ± 0.22	0.894	0.732	0.746
GLOB (g/L)	21.19 ± 0.67 ^{ab}	21.86 ± 0.34 ^b	20.28 ± 0.87 ^{ab}	19.91 ± 1.04 ^{ab}	18.95 ± 0.44 ^a	0.043	0.007	0.022
CHOL (mmol/L)	5.13 ± 0.10	4.97 ± 0.11	5.13 ± 0.08	5.10 ± 0.08	4.93 ± 0.14	0.525	0.426	0.662
TG (mmol/L)	1.58 ± 0.06	1.42 ± 0.03	1.45 ± 0.01	1.54 ± 0.08	1.44 ± 0.02	0.139	0.357	0.420
ALP (U/L)	961.52 ± 14.69 ^a	951.67 ± 4.74 ^a	1016.07 ± 5.42 ^b	952.05 ± 6.44 ^a	982.95 ± 14.98 ^a	0.001	0.336	0.428
LZM (U/L)	148.34 ± 1.41 ^b	135.56 ± 2.38 ^a	128.43 ± 2.55 ^a	130.65 ± 1.86 ^a	128.76 ± 2.74 ^a	0.021	0.006	0.055
ALT (U/L)	26.81 ± 0.52 ^{ab}	27.34 ± 0.59 ^{ab}	26.36 ± 0.13 ^a	28.99 ± 0.52 ^c	27.98 ± 0.54 ^{bc}	0.010	0.038	0.113
AST (U/L)	154.39 ± 5.05 ^a	161.61 ± 2.14 ^{ab}	154.48 ± 3.22 ^a	168.93 ± 4.99 ^b	162.92 ± 3.84 ^{ab}	0.049	0.094	0.250

Data are presented as the mean ± SEM (*n* = 6). Values without a common superscript differ significantly (*P* < 0.05). TP: total protein; ALB: albumin; GLOB: globulin; CHOL: cholesterol; TG: triglycerides; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

TABLE 5: Effects of different substitution levels of BSFM for FM in diets on hepatic nonspecific immunity and antioxidation parameters in juvenile Chinese soft-shelled turtle.

Item	D1	D2	D3	D4	D5	<i>P</i> value	Linear	Quadratic
LZM (U/mg of prot)	165.46 ± 3.35 ^b	152.73 ± 2.00 ^a	145.54 ± 2.56 ^a	146.79 ± 2.84 ^a	145.33 ± 2.79 ^a	0.018	0.005	0.079
T-AOC (U/mg of prot)	8.71 ± 0.12 ^a	9.48 ± 0.27 ^b	9.71 ± 0.16 ^b	9.69 ± 0.20 ^b	9.76 ± 0.18 ^b	0.020	0.007	0.004
GSH-Px (U/mg of prot)	411.74 ± 2.43 ^a	478.05 ± 1.64 ^b	489.31 ± 2.59 ^c	478.03 ± 2.11 ^b	481.84 ± 2.70 ^b	0.000	0.000	0.000
T-SOD (U/mg of prot)	142.11 ± 2.61 ^{ab}	137.67 ± 2.52 ^a	149.48 ± 2.57 ^b	147.73 ± 2.33 ^b	148.88 ± 2.74 ^b	0.012	0.011	0.042
CAT (U/mg of prot)	6.09 ± 0.20	6.53 ± 0.25	6.74 ± 0.18	6.71 ± 0.19	6.23 ± 0.29	0.195	0.538	0.046
MDA (nmol/mg of prot)	9.27 ± 0.12 ^b	7.53 ± 0.24 ^a	7.32 ± 0.08 ^a	7.26 ± 0.22 ^a	7.16 ± 0.09 ^a	0.000	0.000	0.000

Data are presented as the mean ± SEM (*n* = 6). Values without a common superscript differ significantly (*P* < 0.05). LZM: lysozyme; T-AOC: total antioxidant capacity; GSH-Px: glutathione peroxidase; T-SOD: total superoxide dismutase; CAT: catalase; MDA: malonaldehyde.

diet. It was reported that high dietary BSFM levels had adverse effects on the growth rate and FCR of certain species of fish such as the Siberian sturgeon (50% BSFM level; [26]) and Atlantic salmon (25% BSFM level; [27]). Kroeckel et al. [28] found a reduction in the growth performance of fish fed with high levels of BSFM, which could be related to poor feed intake. In this study, all groups showed a similar feed intake, so the compromised growth performance of turtles was probably responsible by the presence of chitin, an inhibitor of nutrient absorption, in BSFM. High dietary levels of BSFM may induce high chitin content, which probably interfere with the digestive process of nutrients [26]. The nutrients in BSFM are coated by chitin; however, fish lack chitinase and cannot digest chitin [29], which results in a decrease in feed efficiency with a subsequent impairment of growth performance.

In the current study, intestinal amylase and protease activities significantly increased in the turtles ingested with 10% to 20% BSFM-containing diets when compared with the other diets. These findings indicate that the digestive enzyme activities of turtles can be increased by replacing FM with 10–20% levels of BSFM in the diet. This result was not consistent with the intestinal activities of trypsin or amylase in rice field eels, which did not significantly differ after feeding the eels with 0, 5.26%, 10.52%, and 15.78% BSFM-containing diets [6]. A reasonable explanation may be the fact that the percentages of BSFM used were different;

chitin is not conducive to the digestion of carbohydrates and protein, and there is a necessity for the body to secrete more amylase and protease to assist with digestion [30]. Moreover, we herein observed a significantly higher activity of lipase in the turtles fed with 5% and 10% BSFM-containing diets than those fed with 20% BSFM-containing diet. However, lipase activity increased in the intestines of rice field eels with increasing levels of dietary BSFM [6]. Lipids or fatty acids act as signalling molecules to modulate certain pathways associated with lipid metabolism such as lipase secretion in the body (Papackova et al., 2015 [31]). Notably, Sánchez-Muros et al. [32] indicated that BSFM cannot be used at a high substitution level because of unbalanced fatty acid compositions. In this study, the imbalance of the fatty acid compositions in the diet could be due to the addition of 20% BSFM that decreased lipase activity.

Serum TP and GLOB levels can reflect the utilization and metabolism of proteins [33]. In this study, serum TP and GLOB levels in the turtles significantly decreased with increasing BSFM levels, indicating an impairment of the digestion and metabolism of proteins in turtles following the substitution of FM with high levels of BSFM in diets. The activity of serum ALP significantly increased in the 10% BSFM diet group, which was not consistent with the study of Wang et al. [9], who observed that replacement of FM with defatted BSFM in diet had no significant impact on serum ALP activity in juvenile Japanese seabass. ALP

TABLE 6: Effects of different substitution levels of BSFM for FM in diets on muscular proximate and amino acid composition (% wet weight) in juvenile Chinese soft-shelled turtles.

Item	D1	D2	D3	D4	D5	P value	Linear	Quadratic
Proximate composition								
Moisture	81.66 ± 0.44	81.79 ± 0.26	81.73 ± 0.34	81.79 ± 0.34	81.60 ± 0.34	0.998	0.861	0.942
Crude protein	16.42 ± 0.10 ^{ab}	16.35 ± 0.05 ^a	16.64 ± 0.08 ^c	16.67 ± 0.05 ^{bc}	16.53 ± 0.07 ^{abc}	0.017	0.055	0.057
Crude fat	0.26 ± 0.01 ^a	0.27 ± 0.01 ^b	0.33 ± 0.01 ^c	0.34 ± 0.01 ^c	0.38 ± 0.01 ^d	0.000	0.000	0.000
Ash	0.65 ± 0.01 ^b	0.60 ± 0.01 ^{ab}	0.61 ± 0.01 ^{ab}	0.56 ± 0.02 ^a	0.56 ± 0.01 ^a	0.000	0.000	0.000
EAA								
Lys	6.60 ± 0.06	6.55 ± 0.05	6.70 ± 0.02	6.73 ± 0.13	6.80 ± 0.10	0.445	0.067	0.190
Met	2.00 ± 0.05	2.13 ± 0.03	2.10 ± 0.02	2.12 ± 0.05	2.16 ± 0.03	0.436	0.395	0.376
Phe	2.97 ± 0.03 ^b	2.59 ± 0.03 ^a	2.53 ± 0.01 ^a	3.61 ± 0.06 ^a	3.66 ± 0.03 ^a	0.019	0.004	0.005
Thr	3.60 ± 0.02	3.73 ± 0.02	3.65 ± 0.02	3.66 ± 0.02	3.76 ± 0.06	0.919	0.825	0.976
Ile	2.67 ± 0.04 ^a	3.03 ± 0.04 ^b	2.99 ± 0.05 ^b	3.06 ± 0.06 ^b	3.00 ± 0.06 ^b	0.000	0.000	0.000
Leu	5.23 ± 0.06	5.19 ± 0.03	5.12 ± 0.05	5.20 ± 0.04	5.13 ± 0.04	0.297	0.504	0.114
Val	4.06 ± 0.04	4.15 ± 0.07	4.24 ± 0.10	4.19 ± 0.06	4.10 ± 0.08	0.246	0.051	0.133
NEAA								
Asp	9.10 ± 0.10	9.00 ± 0.09	8.95 ± 0.07	9.09 ± 0.04	8.91 ± 0.08	0.532	0.177	0.345
Ser	3.90 ± 0.04 ^{ab}	3.93 ± 0.05 ^b	3.62 ± 0.03 ^a	3.55 ± 0.03 ^a	3.99 ± 0.05 ^b	0.008	0.934	0.049
Glu	10.25 ± 0.03 ^c	10.03 ± 0.02 ^{bc}	9.99 ± 0.02 ^{abc}	9.89 ± 0.04 ^a	9.96 ± 0.02 ^{ab}	0.012	0.001	0.003
Gly	3.66 ± 0.03	3.59 ± 0.03	3.63 ± 0.02	3.56 ± 0.04	3.57 ± 0.03	0.122	0.088	0.081
Ala	4.04 ± 0.03	4.10 ± 0.03	4.02 ± 0.03	4.06 ± 0.04	4.03 ± 0.01	0.278	0.200	0.244
Try	2.68 ± 0.02 ^b	2.43 ± 0.02 ^a	2.33 ± 0.02 ^a	2.37 ± 0.02 ^a	2.45 ± 0.02 ^a	0.001	0.002	0.000
His	3.54 ± 0.06	3.56 ± 0.03	3.49 ± 0.04	3.48 ± 0.06	3.58 ± 0.05	0.122	0.068	0.043
Arg	5.67 ± 0.02 ^c	5.39 ± 0.02 ^b	5.35 ± 0.04 ^b	5.20 ± 0.02 ^a	5.19 ± 0.06 ^a	0.000	0.000	0.000
Pro	2.00 ± 0.02 ^a	2.01 ± 0.02 ^a	2.06 ± 0.03 ^{ab}	2.16 ± 0.02 ^b	2.17 ± 0.04 ^b	0.011	0.057	0.113

Data are presented as the mean ± SEM ($n = 6$). Values without a common superscript differ significantly ($P < 0.05$). EAA: essential amino acids such as lysine (Lys), phenylalanine (Phe), methionine (Met), threonine (Thr), isoleucine (Ile), leucine (Leu), and valine (Val); NEAA: nonessential amino acids such as aspartic acid (Asp), serine (Ser), glutamic (Glu), glycine (Gly), alanine (Ala), tryptophan (Try), histidine (His), arginine (Arg), and proline (Pro).

plays an important role in nonspecific immunity by degrading certain pathogens and exogenous proteins [34]. BSFM contains antibacterial peptides, lysozyme, and other bioactive substances [35], and these substances can resist bacteria, inhibit inflammation, enhance immunity, and prevent various stress reactions from damaging the animal body. Therefore, adding an appropriate level of BSFM to the diet may have a certain protective effect on the liver. Serum ALT and AST activities reflect the liver function of animals, and increased activities may be related to liver injury [36]. ALT is also an important transaminase in animals [37]. Serum ALT and AST activities in the 15% BSFM diet group were increased when compared with the 0, 5%, and 10% diet groups. This result was similar to the report of Madibana et al. [38], who conducted replacement of FM with BSFM in diets of juvenile dusky kob (*Argyrosomus japonicus*). These results suggested that the addition of 15% BSFM to the diet affected the liver health of the turtles, but the reasons need to be studied further.

In aquatic animals, health status is generally considered to be closely related to their antioxidant stress capacity. It has been reported that the substitution of FM with BSFM in fish diets could enhance the antioxidant properties by

increasing the activities of certain antioxidant enzymes (T-SOD and GSH-Px) along with T-AOC (Li et al., 2017; [23, 39–41]). In this study, hepatic GSH-Px and T-SOD activities together with T-AOC significantly augmented in the 10%, 15%, and 20% BSFM diet groups. Generally, MDA serves as an important lipid peroxidative product [42]. In this study, the BSFM diet groups (5–20%) showed increased T-AOC coupled with significantly decreased MDA level, which further revealed an enhancement of the antioxidant capacity in turtles fed with BSFM-included diets. This could be supported by the fact that a large amount of chitin and its derivatives that exist in insects have antioxidative activities that can protect against the detriments induced by several diseases [28, 43]. Furthermore, excess PUFA intake can increase oxidative stress and lipid peroxidation in blood and liver tissue of fishes [44]. In this study, with the increase of dietary BSFM levels, PUFA in the diets decreased.

Protein sources and levels in diets can easily cause differences in dietary AAs and fatty acids, which directly affect the body composition of cultured aquatic animals [45]. Herein, we observed that the levels of crude protein and fat in the muscle of turtles linearly elevated with the increase of dietary BSFM levels, whereas the opposite was true for ash content.

Similarly, Chen et al. [46] found that increasing BSFM levels in diets increased the crude protein level in the muscle of juvenile yellow catfish. Moreover, it was also reported that increased levels of dietary BSFM could increase crude fat content and decrease ash content in certain aquatic animals (rainbow trout and Siberian sturgeon) [26, 47]. These findings could be responsible by that BSFM is part of the natural diet of turtles, and the BSFM utilization rate is high in turtles. However, no differences were noted in the effects of the inclusion of dietary BSFM or defatted BSFM on the body composition of various fishes such as the Japanese seabass [9], *Cyprinus carpio* var. Jian [48], *Argyrosomus regius* [49], and the Pacific white shrimp [13]. The discrepancies in the results between the present and previous studies might be due to species specificity. Another reasonable explanation might be that the BSF larvae were raised using food waste in this study, and the nutritional value of BSFM may be higher than that of livestock and poultry waste [50].

AA composition and ratio are important parameters for evaluating the nutritional value of proteins [51]. In this study, replacement of FM with BSFM in diets impacted the AA compositions of turtle muscle to a degree; for example, muscular Phe, Glu, Try, and Arg contents were significantly decreased in the BSFM feeding groups when compared with the FM feeding group. A reasonable explanation for this may be the low Phe, Glu, Try, and Arg contents in BSFM [52], which further suggests that the AA profiles of muscles in aquatic animals reflect dietary AA composition. In addition, the utilization rate of AAs significantly decreased in response to the increase of dietary BSFM levels, which is probably due to that the AAs in BSFM could bind to chitin and might not be digestible [27].

5. Conclusion

Replacing 10% FM by BSFM in the diet of juvenile Chinese soft-shelled turtles elicits improvements on the antioxidant capacity, intestinal digestive enzyme activities, and muscle biochemical indices without impact on the growth performance. Based on the result of broken-line regression analysis of SGR, the optimal replacement level of FM with BSFM in the diets of juvenile Chinese soft-shelled turtles was estimated at 5.0%.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

None of the authors had any conflict of interest.

Authors' Contributions

Rongsheng Shang and Limin Man contributed equally to this work, and all authors agreed to submit the final version of the manuscript.

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References

- [1] P. Alexander, C. Brown, A. Arneth et al., "Could consumption of insects, cultured meat or imitation meat reduce global agricultural land use?," *Global Food Security*, vol. 15, pp. 22–32, 2017.
- [2] K. Nesic and J. Zagon, "Insects - a promising feed and food protein source?," *Meat Technology*, vol. 60, no. 1, pp. 56–67, 2019.
- [3] IPIFF, "Edible insects on the European market," 2021, <http://ipiff.org/wp-content/uploads/2020/06/10-06-2020-IPIFF-edible-insects-market-factsheet.Pdf>.
- [4] M. Henry, L. Gasco, G. Piccolo, and E. Fountoulaki, "Review on the use of insects in the diet of farmed fish: past and future," *Animal Feed Science and Technology*, vol. 203, pp. 1–22, 2015.
- [5] S. Nogales-Merida, P. Gobbi, D. Józefiak et al., "Insect meals in fish nutrition," *Reviews in Aquaculture*, vol. 11, pp. 1080–1103, 2019.
- [6] Y. Hu, Y. Huang, T. Tang et al., "Effect of partial black soldier fly (*Hermetia illucens* L.) larvae meal replacement of fish meal in practical diets on the growth, digestive enzyme and related gene expression for rice field eel (*Monopterus albus*)," *Aquaculture Reports*, vol. 17, article 100345, 2020.
- [7] P. S. Agbohessou, S. N. M. Mandiki, A. Gougbedji et al., "Total replacement of fish meal by enriched-fatty acid *Hermetia illucens* meal did not substantially affect growth parameters or innate immune status and improved whole body biochemical quality of Nile tilapia juveniles," *Aquaculture Nutrition*, vol. 27, no. 3, pp. 880–896, 2021.
- [8] M. Rawski, J. Mazurkiewicz, B. Kierończyk, and D. Józefiak, "Black soldier fly full-fat larvae meal as an alternative to fish meal and fish oil in Siberian sturgeon nutrition: the effects on physical properties of the feed, animal growth performance, and feed acceptance and utilization," *Animals*, vol. 10, no. 11, p. 2119, 2020.
- [9] G. Wang, K. Peng, J. Hu et al., "Evaluation of defatted black soldier fly (*Hermetia illucens* L.) larvae meal as an alternative protein ingredient for juvenile Japanese seabass (*Lateolabrax japonicus*) diets," *Aquaculture*, vol. 507, pp. 144–154, 2019.
- [10] I. Belghit, N. S. Liland, R. Waagbø et al., "Potential of insect-based diets for Atlantic salmon (*Salmo salar*)," *Aquaculture*, vol. 491, pp. 72–81, 2018.
- [11] A. C. Elia, M. T. Capucchio, B. Caldaroni et al., "Influence of *Hermetia illucens* meal dietary inclusion on the histological traits, gut mucin composition and the oxidative stress biomarkers in rainbow trout (*Oncorhynchus mykiss*)," *Aquaculture*, vol. 496, pp. 50–57, 2018.
- [12] R. Magalhães, A. Sánchez-López, R. S. Leal, S. Martínez-Llorens, A. Oliva-Teles, and H. Peres, "Black soldier fly (*Hermetia illucens*) pre-pupae meal as a fish meal replacement in diets for

- European seabass (*Dicentrarchus labrax*)," *Aquaculture*, vol. 476, pp. 79–85, 2017.
- [13] V. C. Cummins, S. D. Rawles, K. R. Thompson et al., "Evaluation of black soldier fly (*Hermetia illucens*) larvae meal as partial or total replacement of marine fish meal in practical diets for Pacific white shrimp (*Litopenaeus vannamei*)," *Aquaculture*, vol. 473, pp. 337–344, 2017.
  - [14] G. Wang, K. Peng, J. Hu, W. Mo, Z. Wei, and Y. Huang, "Evaluation of defatted *Hermetia illucens* larvae meal for *Litopenaeus vannamei*: effects on growth performance, nutrition retention, antioxidant and immune response, digestive enzyme activity and hepatic morphology," *Aquaculture Nutrition*, vol. 27, no. 4, pp. 986–997, 2021.
  - [15] U. Fritz, S. Gong, M. Auer, G. Kuchling, N. Schneeweiß, and A. Hundsdoerfer, "The world's economically most important chelonians represent a diverse species complex (Testudines: Trionychidae: *Pelodiscus*)," *Organisms Diversity & Evolution*, vol. 10, no. 3, pp. 227–242, 2010.
  - [16] C. Y. Li, J. Yang, W. Song, J. Ren, C. Fang, and G. Qian, "Incubation temperature affects the development of calipash related traits and collagen deposition in embryos of soft-shelled turtle *Pelodiscus sinensis*," *Aquaculture*, vol. 528, article 735546, 2020.
  - [17] X. Zhang, Z. Yang, D. Deng et al., "Effects of dietary starch levels on the growth, plasma metabolites and expression of genes involved in glucose transport and metabolism in *Pelodiscus sinensis* juveniles," *Aquaculture Research*, vol. 51, no. 2, pp. 738–750, 2020.
  - [18] Y. Zhong, Y. Pan, L. Liu et al., "Effects of high fat diet on lipid accumulation, oxidative stress and autophagy in the liver of Chinese softshell turtle (*Pelodiscus sinensis*)," *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, vol. 240, article 110331, 2020.
  - [19] F. Zhou, X. Ding, H. Feng et al., "The dietary protein requirement of a new Japanese strain of juvenile Chinese soft shell turtle, *Pelodiscus sinensis*," *Aquaculture*, vol. 412–413, pp. 74–80, 2013.
  - [20] C. X. Sun, D. D. Zhang, W. B. Liu et al., "Growth performance, digestion and metabolism to fish meal replacement by rice protein concentrate in Chinese soft-shelled turtle *Pelodiscus sinensis*," *Aquaculture*, vol. 492, pp. 321–326, 2018.
  - [21] F. Zhou, Y. Wang, X. Ding, W. Ng, F. He, and H. Xue, "Partial replacement of fish meal by soy protein concentrates in diets for a new Japanese strain of juvenile soft-shelled turtle, *Pelodiscus sinensis*," *Aquaculture Research*, vol. 47, no. 3, pp. 875–886, 2016.
  - [22] Y. Zou, Q. Ai, K. Mai, W. Zhang, Y. Zhang, and W. Xu, "Effects of brown fish meal replacement with fermented soybean meal on growth performance, feed efficiency and enzyme activities of Chinese soft-shelled turtle, *Pelodiscus sinensis*," *Journal of Ocean University of China*, vol. 11, no. 2, pp. 227–235, 2012.
  - [23] M. M. Li, S. S. Zhai, Q. Xie et al., "Effects of dietary n-6: n-3 PUFA ratios on lipid levels and fatty acid profile of cherry valley ducks at 15–42 days of age," *Journal of Agricultural and Food Chemistry* 65, vol. 46, pp. 9995–10002, 2017.
  - [24] A. Hamzeh, M. Moslemi, M. Karaminasab, M. A. Khanlar, and R. Tahergorabi, "Amino acid composition of roe from wild and farmed Beluga sturgeon (*Huso huso*)," *Journal of Agricultural Science and Technology*, vol. 17, no. 2, pp. 357–364, 2015.
  - [25] M. Li, M. Zhu, W. Chai et al., "Determination of lipid profiles of Dezhou donkey meat using an LC-MS-based lipidomics method," *Journal of Food Science*, vol. 86, no. 10, pp. 4511–4521, 2021.
  - [26] C. Caimi, M. Renna, C. Lussiana et al., "First insights on Black Soldier Fly (*Hermetia illucens* L.) larvae meal dietary administration in Siberian sturgeon (*Acipenser baerii* Brandt) juveniles," *Aquaculture*, vol. 515, article 734539, 2020.
  - [27] P. Weththasinghe, J. Hansen, D. Nøklund, L. Lagos, M. Rawski, and M. Øverland, "Full-fat black soldier fly larvae (*Hermetia illucens*) meal and paste in extruded diets for Atlantic salmon (*Salmo salar*): effect on physical pellet quality, nutrient digestibility, nutrient utilization and growth performances," *Aquaculture*, vol. 530, article 735785, 2021.
  - [28] S. Kroeckel, A. G. E. Harjes, I. Roth et al., "When a turbot catches a fly: evaluation of a pre-pupae meal of the black soldier fly (*Hermetia illucens*) as fish meal substitute—growth performance and chitin degradation in juvenile turbot (*Psetta maxima*)," *Aquaculture*, vol. 364–365, pp. 345–352, 2012.
  - [29] S. Shiau and Y. Yu, "Dietary supplementation of chitin and chitosan depresses growth in tilapia, *Oreochromis niloticus* × *O. aureus*," *Aquaculture*, vol. 179, no. 1–4, pp. 439–446, 1999.
  - [30] S. Marono, G. Piccolo, R. Loponte et al., "In vitro crude protein digestibility of *Tenebrio molitor* and *Hermetia illucens* insect meals and its correlation with chemical composition traits," *Italian Journal of Animal Science*, vol. 14, no. 3, p. 3889, 2015.
  - [31] Z. Papackova and M. Cahova, "Fatty acid signaling: the new function of intracellular lipases," *International Journal of Molecular Sciences* 16, no. 2, pp. 3831–3855, 2015.
  - [32] M. Sánchez-Muros, C. de Haro, A. Sanz, C. E. Trenzado, S. Villareces, and F. G. Barroso, "Nutritional evaluation of *Tenebrio molitor* meal as fishmeal substitute for tilapia (*Oreochromis niloticus*) diet," *Aquaculture Nutrition*, vol. 22, no. 5, pp. 943–955, 2016.
  - [33] J. Huang, W. Sun, C. Li et al., "Effects of DL-methionine supplement on growth performance and amino acid digestion and plasma concentrations in sika deer calves (*Cervus nippon*)," *Animal Production Science*, vol. 56, no. 6, pp. 1002–1007, 2016.
  - [34] R. P. Ellis, H. Parry, J. I. Spicer, T. H. Hutchinson, R. K. Pipe, and S. Widdicombe, "Immunological function in marine invertebrates: responses to environmental perturbation," *Fish & Shellfish Immunology*, vol. 30, no. 6, pp. 1209–1222, 2011.
  - [35] O. Elhag, D. Zhou, Q. Song et al., "Screening, expression, purification and functional characterization of novel antimicrobial peptide genes from *Hermetia illucens* (L.)," *PLoS One*, vol. 12, no. 1, article e0169582, 2017.
  - [36] K. I. Dorcas and J. Solomon, "Calculation of liver function test in *Clarias gariepinus* collected from three commercial fish ponds," *Nature and Science*, vol. 12, no. 10, pp. 107–123, 2014.
  - [37] J. Huang, T. Zhang, B. Kun, G. Li, and K. Wang, "Effect of supplementation of lysine and methionine on growth performance, nutrients digestibility and serum biochemical indices for growing sika deer (*Cervus nippon*) fed protein deficient diet," *Italian Journal of Animal Science*, vol. 14, no. 1, p. 3640, 2015.
  - [38] M. Madibana, M. Mwanza, B. Lewis, C. Fouché, R. Toefy, and V. Mlambo, "Black soldier fly larvae meal as a fishmeal substitute in juvenile dusky kob diets: effect on feed utilization, growth performance, and blood parameters," *Sustainability*, vol. 12, no. 22, p. 9460, 2020.
  - [39] J. R. Hu, G. X. Wang, W. Y. Mo et al., "Effects of fish meal replacement by black soldier fly (*Hermetia illucens* L.) larvae meal on growth performance, body composition, plasma

- biochemical indexes and tissue structure of juvenile *Lateolabrax japonicus*,” *Chinese Journal of Animal Nutrition*, vol. 11, no. 31, pp. 5292–5300, 2018.
- [40] X. Xiao, P. Jin, L. Zheng et al., “Effects of black soldier fly (*Hermetia illucens*) larvae meal protein as a fishmeal replacement on the growth and immune index of yellow catfish (*Pelteobagrus fulvidraco*),” *Aquaculture Research*, vol. 49, no. 4, pp. 1569–1577, 2018.
- [41] X. Xu, H. Ji, H. Yu, and J. Zhou, “Influence of dietary black soldier fly (*Hermetia illucens* Linnaeus) pulp on growth performance, antioxidant capacity and intestinal health of juvenile mirror carp (*Cyprinus carpio* var. *Specularis*),” *Aquaculture Nutrition*, vol. 26, no. 2, pp. 432–443, 2020.
- [42] M. Koruk, S. Taysi, M. Savaş, O. Yilmaz, F. Akcay, and M. Karakok, “Oxidative stress and enzymatic antioxidant status in patients with nonalcoholic steatohepatitis,” *Annals of Clinical and Laboratory Science*, vol. 34, no. 1, pp. 57–62, 2004.
- [43] D. Ngo and S. Kim, “Chapter two - antioxidant effects of chitin, chitosan, and their derivatives,” in *Advances in Food and Nutrition Research*, S. Kim, Ed., pp. 15–31, Academic Press, 2014.
- [44] M. Li, C. Xu, Y. Ma et al., “Effects of dietary n-3 highly unsaturated fatty acids levels on growth, lipid metabolism and innate immunity in juvenile golden pompano (*Trachinotus ovatus*),” *Fish & Shellfish Immunology*, vol. 105, pp. 177–185, 2020.
- [45] Y. Ma, M. Li, D. Xie et al., “Fishmeal can be replaced with a high proportion of terrestrial protein in the diet of the carnivorous marine teleost (*Trachinotus ovatus*),” *Aquaculture*, vol. 519, article 734910, 2020.
- [46] X. Chen, J. Hu, G. Wang et al., “Effects of fish meal replacement by black soldier fly (*Hermetia illucens*) larvae meal on growth performance, serum biochemical indices and meat quality of juvenile yellow catfish (*Pelteobagrus fulvidraco*),” *Chinese Journal of Animal Nutrition*, vol. 31, no. 6, pp. 2788–2799, 2019.
- [47] M. Renna, A. Schiavone, F. Gai et al., “Evaluation of the suitability of a partially defatted black soldier fly (*Hermetia illucens* L.) larvae meal as ingredient for rainbow trout (*Oncorhynchus mykiss* Walbaum) diets,” *Journal of Animal Science and Biotechnology*, vol. 8, no. 1, p. 57, 2017.
- [48] Y. Emre, A. Kurtoglu, N. Emre, B. Güroy, and D. Güroy, “Effect of replacing dietary fish oil with soybean oil on growth performance, fatty acid composition and haematological parameters of juvenile meagre, *Argyrosomus regius*,” *Aquaculture Research*, vol. 47, no. 7, pp. 2256–2265, 2016.
- [49] I. Guerreiro, C. Castro, B. Antunes et al., “Catching black soldier fly for meagre: growth, whole-body fatty acid profile and metabolic responses,” *Aquaculture*, vol. 516, article 734613, 2020.
- [50] M. A. El-Dakar, R. R. Ramzy, M. Plath, and H. Ji, “Evaluating the impact of bird manure vs. mammal manure on *Hermetia illucens* larvae,” *Journal of Cleaner Production*, vol. 278, article 123570, 2021.
- [51] J. D. Bihuniak and K. L. Insogna, “The effects of dietary protein and amino acids on skeletal metabolism,” *Molecular and Cellular Endocrinology*, vol. 410, pp. 78–86, 2015.
- [52] M. M. Li, M. F. Li, G. Wang et al., “Defatted black soldier fly (*Hermetia illucens*) larvae meal can partially replace fish meal in diets for adult Chinese soft-shelled turtles,” *Aquaculture*, vol. 541, article 736758, 2021.