

Research Article

Effects of Fermented Soybean Meal Substituting Plant Protein and Fish Meal on Growth, Flesh Quality, and Intestinal Microbiota of Largemouth Bass (*Micropterus salmoides*)

Beibei Guo,^{1,2,3} Lingling Huang,⁴ Xiaoqin Li,^{1,2,3} Yunfeng Chen,^{1,2,3} Tianyu Huang,^{1,2,3} Lizhou Ma,⁴ and Xiangjun Leng^{id}^{1,2,3}

¹National Demonstration Center for Experimental Fisheries Science Education, Shanghai Ocean University, Shanghai, China

²Centre for Research on Environmental Ecology and Fish Nutrition (CREEFN) of the Ministry of Agriculture, Shanghai Ocean University, Shanghai, China

³Shanghai Collaborative Innovation for Aquatic Animal Genetics and Breeding, Shanghai Ocean University, Shanghai, China

⁴Wilmar (Shanghai) Biotechnology Research & Development Center Co. Ltd., China

Correspondence should be addressed to Xiangjun Leng; xjleng@shou.edu.cn

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This study investigated the effects of replacing soybean meal (SM), corn gluten meal (CGM), and fish meal (FM) with fermented soybean meal (FSM) on growth performance, flesh quality, and intestinal microbiota of largemouth bass. In a diet with 400 g/kg FM inclusion (FSM-0), FSM was used to substitute 120 g/kg SM, 120 g/kg SM + 80 g/kg CGM, and 65 g/kg FM at the inclusion of 100 g/kg (FSM-10-SM), 200 g/kg (FSM-20), and 100 g/kg (FSM-10-FM), respectively. Then, largemouth bass weighing 225.7 ± 2.6 g was fed the four diets for 60 days. The WG of FSM-20 group was increased by 11.8% ($P < 0.05$), while FSM-10-SM and FSM-10-FM groups did not differ significantly from FSM-0 group in growth performance. Compared with the FSM-0 group, the flesh hardness, shear force, total collagen, hot soluble collagen, and total free amino acid contents of FSM-20 group were significantly increased, and FSM-10-SM group also presented higher flesh hardness and shear force. The flesh superoxide dismutase (SOD) and total antioxidant capacity (TAOC) activities in FSM-20 and FSM-10-FM groups and glutathione peroxidase (GSH-Px) activity in FSM-10-FM group were increased ($P < 0.05$), while the lactic acid content in FSM-20 group was decreased, when compared to FSM-0 group ($P < 0.05$). In serum biochemical indexes, the glucose, total protein, cholesterol and malondialdehyde contents in the three FSM groups, and the triglyceride content in FSM-10-SM and FSM-20 groups were significantly lower than those of FSM-0 group ($P < 0.05$). In intestinal histology, the intestinal villus width, muscle thickness in FSM-10-SM group and intestinal villus width in FSM-20 group were significantly higher than those of FSM-0 group. In intestinal microbiota, the three FSM groups showed higher abundance of Firmicutes and lower abundance of Proteobacteria than FSM-0 group. In summary, the replacement of 120 g/kg SM or 65 g/kg FM with 100 g/kg FSM (FSM-10-SM and FSM-10-FM groups) did not exhibit significant effect on growth performance, but partially improved the flesh quality of largemouth bass. The substitution of 120 g/kg SM + 80 g/kg CGM with 200 g/kg FSM (FSM-20 group) significantly improved weight gain and flesh quality of largemouth bass.

1. Introduction

Soybean meal (SM) is the most commonly used plant protein source in aquatic feeds. However, antinutritional factors found in SM have limited its extensive use in aquatic feeds, which contain trypsin inhibitors, nonstarch polysaccharides, oligosaccharides, phytates, soybean antigens, and saponins, etc. [1]. These antinutritional factors have been reported to

reduce feed intake [2], cause intestinal mechanical damage, and reduce intestinal microbial diversity and digestive enzyme activity [3–5], adversely impacting ingestion and absorption of aquatic animals.

Microbial fermentation is an effective method for promoting the nutritional value of SM [6]. During fermentation, microorganisms cannot only effectively degrade antinutritional factors such as nonstarch polysaccharides, oligosaccharides

[7], trypsin inhibitors [8], and soybean antigenic proteins in SM, but also increase the contents of organic acids [9], free amino acids [6], soybean active peptides, polyphenols [10], and other functional substances. In the study of Li et al. [11], Largemouth bass (*Micropterus salmoides*) fed with mixed microbial fermented SM showed higher specific gain rate and serum lysozyme activity than fed with SM, and FSM also improved intestinal lesions caused by SM. In turbot (*Scophthalmus maximus* L.), dietary FSM (*Lactobacillus acidophilus* fermentation) significantly increased feed intake, weight gain, digestive- and immune-related enzyme activities, and reduced SM-induced intestinal lesions and positively regulated the intestinal microbiota [12]. Wang et al. [13] reported that *Lactobacillus plantarum* fermented SM could substitute 45% of dietary FM for juvenile turbot, while the replaced ratio of FM by SM was only 30%. Currently, the research of FSM in aquatic animals concentrated on growth performance, antioxidant, and intestinal health but less on flesh quality.

In Japanese seabass (*Lateolabrax japonicus*), with the supplementation of essential amino acids, the replacement of 180 g/kg FM with multimicroorganism fermented SM had no adverse effects on protein, lipid contents, and fatty acid composition in dorsal muscles but decreased the flesh hardness and chewiness [14]. Rahimnejad et al. [15] used *Bacillus pumilus* fermented SM to substitute FM in the diet of Japanese seabass, and no significant effects were found on muscle moisture, protein, and lipid contents, but crude ash content decreased with the increasing FSM level. In rockfish (*Sebastes schlegeli*), the proximate and essential amino acid composition of muscle did not differ significantly when *Bacillus subtilis* fermented SM was used as a dietary replacement for FM [16]. However, limited previous research has been conducted to investigate the effects of FSM inclusion on flesh quality properties such as texture and flavor compounds.

At present, largemouth bass has been widely cultured in many countries due to its rapid growth, strong disease resistance, and delicious meat. In 2021, the aquaculture production of this fish reached 702,093 tons in China (China Fisheries Yearbook, 2022). Currently, the replacement of FM with FSM has been reported in largemouth bass [17, 18] but not for plant protein. In addition, most of the prior studies used juvenile fish, focusing on the growth, intestine, and immunity but few on flesh quality such as texture and flavor compounds. Therefore, in this study, FSM was applied to substitute plant protein (SM, corn gluten meal (CGM), or fish meal (FM)); the impacts of this substitution were investigated on the growth performance, flesh quality, and intestinal health of large-size largemouth bass. The results will supply a theoretical foundation for applying FSM in carnivorous fish feed.

2. Materials and Methods

In the present study, all experimental animal care protocols were approved by the Institutional Animal Care and Use Committee (IACUC). All animal handling procedures performed were strictly followed the Regulations of the

Experimental Animal Ethics Committee of Shanghai Ocean University.

2.1. Experimental Design. First, the control diet was devised to include 400 g/kg of FM, 120 g/kg of SM, and 80 g/kg of CGM. Based on the control diet (FSM-0), 100 g/kg, 200 g/kg, and 100 g/kg FSM were used to replace all SM (120 g/kg), all SM (120 g/kg) + all CGM (80 g/kg) and 65 g/kg FM, respectively to form four isonitrogenous (460 g/kg crude protein) and isolipidic (120 g/kg crude lipid) diets, recorded as FSM-0, FSM-10-SM, FSM-20, and FSM-10-FM. The diets formulation is shown in Table 1. Large particles of raw materials were crashed first and then passed through a 60 mesh sieve. After all the raw materials were mixed evenly, sinking pellets with diameter of 3 mm were produced by a single-screw extruder (extruding temperature, $85 \pm 5^\circ\text{C}$); then, all diets were naturally air-dried and sealed in a cool and dry place. The diets proximate composition is shown in Table 1, and amino acid composition of ingredients and diets is shown in Table 2.

FSM was supplied by Yihai Kerry Biotechnology Co., Ltd. FSM is produced by anaerobic fermentation of SM with lactic acid bacteria. The fermentation temperature, fermentation time, and drying temperature were 30°C , 72 hr, and 55°C , respectively. The crude protein and crude lipid contents of FSM were 500.0 and 20.0 g/kg.

2.2. Experimental Fish and Feeding Management. Largemouth bass was provided by the Binhai Aquaculture Station of Shanghai Ocean University. A total of 120 largemouth bass with initial body weight of 225.7 ± 2.6 g were selected and randomly placed in 12 cages hung in cement pools. All the fish were fed twice daily (8:00 and 16:00), and the feed intake was kept the same (about 1.5% of the body weight) for all cages in the early 2 weeks. Then, the fish were fed to apparent satiation depending on feeding behavior to ensure no feed residue left. About one-third of the water was renewed every 3 days, and the feces at the bottom of cage was removed every 6 days. During the breeding period, temperature electrode and dissolved oxygen electrode were used to monitor water temperature ($27.5 \pm 2.5^\circ\text{C}$) and dissolved oxygen (6.0–7.0 mg/L), and water quality monitoring kits were used to detect pH (7.0–7.5), ammonia nitrogen (≤ 0.2 mg/L), and nitrite (≤ 0.1 mg/L). The feeding trial was implemented at Binhai Aquaculture Station of Shanghai Ocean University, and the feeding period was 60 days.

2.3. Samples Collection. Before the start of the breeding experiment, five fish were randomly preserved for body composition analysis. After 60 days of feeding, the fish weight and quantity in each cage were recorded, and three fish were randomly selected to test the proximate composition of whole body. The other three fish were taken and measured body length and weight and then collected blood. Visceral was dissected to calculate the hepatosomatic index and viscerosomatic index. Intestines were collected to test digestive enzyme activity and tissue sections. Dorsal muscles were collected to detect proximate composition, amino acids composition, collagen, antioxidant parameters, and water holding capacity (WHC). In addition, another three fish per cage

TABLE 1: Formulation and proximate composition of diets (air-dried basis, g/kg).

Ingredients ^a	FSM-0	FSM-10-SM	FSM-20	FSM-10-FM
Fish meal	400	400	400	335
Fermented soybean meal	0	100	200	100
Soybean meal	120	0	0	120
Corn gluten meal	80	80	0	80
Soy protein concentrate	70	70	70	70
Wheat flour	129.5	149.5	129.5	94.5
Wheat gluten	50	50	50	50
Fish oil	20	20	20	20
Soybean oil	25	25	25	25
Soybean lecithin	25	25	25	25
Squid visceral meal	40	40	40	40
Monocalcium phosphate	20	20	20	20
Premix ^b	20	20	20	20
Total	1,000	1,000	1,000	1,000
Proximate composition (g/kg)				
Moisture	86.5	95.7	93.6	93.7
Crude protein	514.3	508.7	510.6	516.3
Crude lipid	118.7	116.7	114.4	110.8
Crude ash	100.2	99.4	108.6	100.3

Note: ^aFish meal, soybean meal, corn gluten meal, and soy protein concentrate were purchased from Zhejiang Hanbei Biological Co., Ltd. (China). The protein contents of fish meal, fermented soybean meal, soy protein concentrate, soybean meal, corn gluten meal, wheat flour, and wheat gluten were 672.2, 500.0, 654.0, 442.0, 570.2, 144.1, and 752.0 g/kg, respectively. ^bPremix was purchased from Zhejiang Hanbei Biological Co., Ltd. (China). Premix contains vitamins and minerals (mg or IU/kg diet): VA, 3,000 IU; VD3, 1,500 IU; VE, 40 mg; VK3, 4.5 mg; VB1, 8 mg; VB2, 8.5 mg; VB6, 6 mg; VB12, 0.015 mg; VC, 110 mg; biotin, 0.15 mg; inositol, 40 mg; folic acid, 1.3 mg; D-calcium pantothenate, 17 mg; I, 1.2 mg; Mn, 8.5 mg; Co, 1 mg; Cu, 6.5 mg; Zn, 53 mg; Se, 0.35 mg; Fe, 45 mg.

were selected, and the white muscle above the lateral line behind the head was used for the measurement of flesh color, texture, and shear force.

2.4. Indicators and Methods

2.4.1. Growth Performance and Physical Indices.

$$\text{Feed intake (FI, g/fish/d)} = \text{FI}(g) / ((\text{FFN} + \text{IFN}) / 2) / \text{days}, \quad (1)$$

$$\text{Weight gain (WG, \%)} = (\text{FBW}(g) - \text{IBW}(g)) / \text{IBW}(g) \times 100, \quad (2)$$

$$\text{Survival (\%)} = (\text{FFN} / \text{IFN}) \times 100, \quad (3)$$

$$\text{Feed conversion ratio (FCR)} = \text{FI}(g) / (\text{FBW}(g) - \text{IBW}(g)), \quad (4)$$

$$\text{Viscerosomatic index (VSI, \%)} = (\text{FVW}(g) / \text{FBW}(g)) \times 100, \quad (5)$$

$$\text{Hepatosomatic index (HSI, \%)} = (\text{FLW}(g) / \text{FBW}(g)) \times 100, \quad (6)$$

$$\text{Condition factor (CF, } gcm^{-3}\text{)} = (\text{FBW}(g) / \text{BL}(cm)^3) \times 100, \quad (7)$$

$$\text{Protein efficiency rate (PER)} = (\text{FBW}(g) - \text{IBW}(g)) / \text{PI}(g), \quad (8)$$

$$\text{Protein retention (PR, \%)} = (\text{FBP}(g) - \text{IBP}(g)) / \text{PI}(g) \times 100, \quad (9)$$

$$\text{Lipid retention (LR, \%)} = (\text{FBL}(g) - \text{IBL}(g)) / \text{LI}(g) \times 100, \quad (10)$$

where FFN refers to final fish number, IFN refers to initial fish number, FBW refers to final body weight, IBW refers to initial body weight, FVW refers to final visceral weight, FLW refers to initial liver weight, BL refers to body length, FBP refers to final body protein, IBP refers to initial body protein, FBL refers to final body lipid, IBL refers to initial body lipid, PI refers to protein intake, and LI refers to lipid intake.

2.4.2. The Diet, Whole-Body and Flesh Proximate Composition. The proximate composition of feed, whole body, and flesh included moisture, crude ash, crude protein, and crude lipid. The analysis methods of the above indicators include high temperature drying method (moisture), high temperature burning method (crude ash), Kjeldahl method (crude protein), and Soxhlet extraction method (crude lipid). Details of the operation steps were obtained from AOAC (2005).

2.4.3. Determination of Amino Acids. To determine amino acids in feed and muscle, the dried samples were hydrolyzed with 6 mol/L hydrochloric acid; they are filtered through a

TABLE 2: Amino acid composition of ingredients and diets (dry matter, g/kg).

Parameters	Fish meal	FSM	FSM-0	FSM-10-SM	FSM-20	FSM-10-FM
Essential amino acids (EAAs)						
Arginine	38.9	33.9	30.8	31.8	31.3	31.2
Histidine	16	12.2	14.3	14.5	15.1	14.7
Isoleucine	30.8	23.6	21.7	22.1	22.2	22.3
Leucine	50	40.5	39.4	39.8	40.1	39.4
Lysine	50.6	29.9	29.7	29.5	28.7	29.4
Methionine	19.4	7.8	11.3	11.5	11.1	10.7
Phenylalanine	27.5	25.6	23.5	24.2	24.6	23.5
Threonine	28.1	19.1	17.8	18.4	18.5	17.9
Valine	34.8	23.1	24.0	24.7	24.6	24.9
Nonessential amino acids (NEAAs)						
Alanine	44.2	22.2	26.3	26.4	26.4	25.8
Aspartic acid	61.0	54.1	45.0	45.2	45.2	44.5
Cysteine	4.1	10.6	6.6	6.5	5.9	7.1
Glutamic acid	87.5	86.9	82.3	83.8	84.9	82.7
Glycine	41.3	20.9	25.7	25.6	25.6	24.8
Serine	26.1	24.0	20.0	20.3	20.5	19.7
Tyrosine	22.9	19.0	13.9	13.6	11.4	14.2
Proline	28.4	24.2	18.3	18.2	17.5	19.6
Total amino acids (TAAs)	611.6	477.6	450.6	456.0	453.4	452.3

0.22- μ m filter membrane and determined on the Sykam S 433 Amino Acid Automatic Analyzer (Sykam, Germany). Free amino acids (FAAs) in muscle were determined with reference to the method by Xu et al. [19]. FAAs were extracted from the samples using 5% trichloroacetic acid solution and determined using an automatic amino acid analyzer (Hitachi LA8080, Japan) after centrifugation and filtration.

2.4.4. Flesh Quality. The lightness (L^*), redness (a^*), and yellowness (b^*) values of the muscles were determined using colorimeter (CR-10, Konica Minolta Co., Ltd., Osaka, Japan). Steaming loss and freezing loss were determined with reference to the methods by Sun et al. [20] and Zhang et al. [21].

Universal TA Texture Analyzer (Tengba Company) was used to measure texture and shear force of the dorsal muscle. The testing conditions of texture were as follows: a flat-bottomed cylindrical probe with size of 25 \times 25 mm, the compression ratio 30%, the test speed 1 mm/s, the recording speed 1 mm/s, and the staying time 2 s. For the measurement of shear force, the speed before the test, the compression distance, the staying time, and the speed after the test were 1 mm/s, 20 mm, 2 s, and 1 mm/s, respectively.

The content of total collagen and heat-soluble collagen in muscle was determined by hydroxyproline kits (Nanjing Jiancheng Biotechnology Institute). Heat-soluble collagen was detected with the description by Kong et al. [22]. The amount of heat-insoluble collagen content is the difference between total collagen and heat-soluble collagen.

2.4.5. Serum and Muscle Biochemical Parameters. Serum and muscle antioxidant indexes were measured, including total antioxidant capacity (TAOC), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA),

cathepsin B (Cath-B), and lactic acid (LA). Serum biochemical parameters included glucose (GLU), total protein (TP), triglyceride (TG), and total cholesterol (CHO). All the measurements were conducted using the kits produced by Nanjing Jiancheng Biotechnology Institute.

2.4.6. Intestinal Digestive Enzyme Activity. Foregut tissue was used to determine protease and amylase activities. The protease activity was determined by Folin phenol method. Amylase activity was determined by starch-iodine colorimetry. They were conducted using the kits produced by Nanjing Jiancheng Biotechnology Institute.

2.4.7. Intestinal Histology. Foregut tissue was used for intestinal tissue section preparation. The foregut was fixed, eluted, transparentized, embedded, sectioned, and stained and then photographed using a microscope (Nikon YS100). Image 14.0 was used to measure intestinal villus height (VH), intestinal villus width (VW), and muscular thickness (MT).

2.4.8. Intestinal Microbiota. After 2 weeks of storing at -80°C , intestinal samples were sent to Shanghai Majorbio Bio-Pharm Technology Co., Ltd. for the determination of intestinal microorganisms by the Illumina MiSeq Sequencing platform. The steps were as follows: DNA extraction uses the kits produced by QIAGEN Biotechnology Institute, amplification of the V3-V4 variable region of the 16S rRNA gene with primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), amplicons purification, and paired-end sequencing (2 \times 300). UPARSE was used to perform operational taxonomic unit (OTUs) clustering analysis based on 97% similarity, and representative sequences of OTUs were obtained. Community abundance and

TABLE 3: Growth performance and physical indices of largemouth bass.

Parameters	FSM-0	FSM-10-SM	FSM-20	FSM-10-FM
IBW (g)	226.6 ± 2.6	224.6 ± 2.6	228.9 ± 2.5	222.7 ± 2.5
FBW (g)	367.7 ± 12.5 ^a	378.3 ± 20.5 ^{ab}	397.2 ± 8.8 ^b	353.2 ± 12.3 ^a
FI (g/fish/d)	2.86 ± 0.11 ^{ab}	2.94 ± 0.09 ^b	3.20 ± 0.10 ^c	2.68 ± 0.03 ^a
WG (%)	62.3 ± 1.1 ^a	68.4 ± 8.1 ^{ab}	73.6 ± 5.4 ^b	58.6 ± 2.0 ^a
FCR	1.18 ± 0.01	1.12 ± 0.10	1.11 ± 0.04	1.19 ± 0.01
SR (%)	100	100	100	100
VSI (%)	7.62 ± 0.47 ^b	7.59 ± 0.56 ^b	6.92 ± 0.33 ^{ab}	6.51 ± 0.37 ^a
HSI (%)	2.23 ± 0.19 ^c	2.20 ± 0.11 ^{bc}	2.01 ± 0.12 ^{ab}	1.81 ± 0.13 ^a
CF (g cm ⁻³)	2.65 ± 0.11 ^b	2.58 ± 0.15 ^{ab}	2.53 ± 0.13 ^{ab}	2.42 ± 0.14 ^a

Note: In the same row, values with the same letter superscripts mean no significant difference ($P > 0.05$), while with different small letter superscripts mean significant difference ($P < 0.05$). IBW, initial body weight; FBW, final body weight; FI, feed intake; WG, weight gain; FCR, feed conversion ratio; SR, survival rate; VSI, viscerosomatic index; HSI, hepatosomatic index; CF, condition factor.

TABLE 4: Whole body and flesh composition of largemouth bass (fresh tissue, g/kg).

Parameters	FSM-0	FSM-10-SM	FSM-20	FSM-10-FM
Whole body				
Moisture	692.6 ± 1.2	691.1 ± 2.0	691.1 ± 7.3	698.2 ± 11.1
Crude protein	171.9 ± 4.2	170.2 ± 2.3	170.7 ± 7.6	171.4 ± 8.2
Crude lipid	62.4 ± 2.2	66.1 ± 4.0	64.5 ± 3.9	66.3 ± 1.0
Crude ash	47.2 ± 1.8	46.7 ± 2.4	47.0 ± 1.3	46.7 ± 4.7
Flesh				
Moisture	772.4 ± 3.3	769.8 ± 4.3	773.4 ± 1.7	769.1 ± 2.5
Crude protein	194.0 ± 0.2	192.7 ± 0.1	194.6 ± 2.7	193.0 ± 2.0
Crude lipid	13.20 ± 0.32	13.70 ± 0.37	13.92 ± 0.13	13.65 ± 0.48
Crude ash	12.4 ± 0.7	12.3 ± 0.3	11.5 ± 1.1	11.8 ± 1.0
Total collagen	2.72 ± 0.13 ^a	3.02 ± 0.21 ^{ab}	3.39 ± 0.42 ^b	2.85 ± 0.14 ^a
Heat-soluble collagen (HS)	1.38 ± 0.08 ^a	1.73 ± 0.15 ^b	2.13 ± 0.10 ^c	1.83 ± 0.14 ^b
Heat-insoluble collagen (HIS)	1.34 ± 0.12 ^b	1.29 ± 0.07 ^b	1.26 ± 0.08 ^b	1.02 ± 0.09 ^a

Note: In the same row, values with the same letter superscripts mean no significant difference ($P > 0.05$), while with different small letter superscripts mean significant difference ($P < 0.05$).

composition at the phylum and genus levels were analyzed on the Majorbio Cloud Platform.

2.5. Statistical Analysis. The test data were expressed as mean ± standard deviation. SPSS 26.0 was used for one-way analysis of variance (ANOVA). In the case of significant difference ($P < 0.05$), Duncan's program was used for multiple comparisons.

3. Results

3.1. Growth Performance and Physical Indices. As shown in Table 3, the replacement of 120 g/kg SM + 80 g/kg CGM with 200 g/kg FSM (FSM-20) increased the FI and WG (+18.13%) ($P < 0.05$) and tended to reduce the FCR ($P = 0.190$). The replacement of 120 g/kg SM or 65 g/kg FM with 100 g/kg FSM showed no significant effects on WG and FCR, but the FSM-10-FM group had significantly lower VSI, HSI, and CF than FSM-0 group ($P < 0.05$).

3.2. Whole Body and Flesh Composition. As shown in Table 4, there were no obvious changes in moisture, crude protein, crude lipid, and crude ash contents in whole fish and flesh among all groups ($P > 0.05$). The total collagen and HS

contents in FSM-20 group were higher than those in the other three groups (except total collagen in FSM-10-SM group) ($P < 0.05$), and the HS contents in FSM-10-SM and FSM-10-FM groups were also higher than those in FSM-0 group ($P < 0.05$), while the HIS content in FSM-10-FM group was lower than that in FSM-0 group ($P < 0.05$).

3.3. Flesh Color, Water-Holding Capacity, and Texture. As shown in Table 5, there were no obvious changes in flesh lightness (L^*), redness (a^*), yellowness (b^*), steaming loss, thawing loss, springiness, and cohesiveness among all the groups ($P > 0.05$). Flesh hardness and shear force of FSM-10-SM and FSM-20 groups and shear force of FSM-10-FM group were higher than FSM-0 group ($P < 0.05$), and the chewiness of FSM-10-FM group was lower than FSM-0 group ($P < 0.05$).

3.4. Flesh Amino Acid. In muscle amino acid composition, there were no obvious changes observed among all of the groups ($P > 0.05$) (Table 6).

As shown in Table 7, FSM-20 group showed higher TFAAs content than FSM-0 group ($P < 0.05$). The contents of histidine, lysine, alanine, aspartic acid, and tyrosine were higher, while arginine content was lower in FSM-10-SM group compared to

TABLE 5: Flesh color, water-holding capacity, texture characteristics, and shear force of largemouth bass.

Parameters	FSM-0	FSM-10-SM	FSM-20	FSM-10-FM
Lightness (L*)	37.28 ± 1.70	37.42 ± 4.01	38.70 ± 2.71	36.95 ± 1.26
Redness (a*)	-1.00 ± 0.18	-1.05 ± 0.41	-1.10 ± 0.34	-1.07 ± 0.21
Yellowness (b*)	9.55 ± 0.59	9.56 ± 0.67	9.66 ± 0.85	9.50 ± 0.78
Steaming loss (%)	9.38 ± 0.06	9.19 ± 1.18	9.05 ± 0.01	9.52 ± 0.86
Thawing loss (%)	3.02 ± 0.40	2.90 ± 0.42	2.70 ± 0.35	3.05 ± 0.44
Hardness (gf)	225.6 ± 15.3 ^a	295.7 ± 22.0 ^b	278.4 ± 20.1 ^b	233.1 ± 22.1 ^a
Springiness	0.45 ± 0.06	0.45 ± 0.06	0.44 ± 0.06	0.42 ± 0.07
Cohesiveness (gf)	0.54 ± 0.09	0.56 ± 0.10	0.55 ± 0.08	0.51 ± 0.06
Chewiness (gf)	99.2 ± 9.9 ^b	98.0 ± 9.1 ^b	98.5 ± 6.4 ^b	79.7 ± 6.2 ^a
Shear force (gf)	2966.9 ± 207.9 ^a	3399.3 ± 290.3 ^b	3430.4 ± 259.5 ^b	3528.0 ± 209.9 ^b

Note: In the same row, values with the same letter superscripts mean no significant difference ($P > 0.05$), while with different small letter superscripts mean significant difference ($P < 0.05$).

TABLE 6: Hydrolyzed amino acid composition of largemouth bass flesh (dry matter basis, g/kg).

Parameters	FSM-0	FSM-10-SM	FSM-20	FSM-10-FM
Arginine	48.8 ± 1.6	48.3 ± 0.7	49.2 ± 3.1	49.1 ± 2.0
Histidine	22.6 ± 1.3	21.4 ± 0.3	22.0 ± 0.4	22.0 ± 0.5
Isoleucine	38.5 ± 2.1	39.0 ± 0.5	39.5 ± 1.7	40.9 ± 1.7
Leucine	65.5 ± 2.0	65.1 ± 0.0	67.1 ± 0.4	67.0 ± 2.0
Lysine	87.3 ± 5.8	88.5 ± 0.2	88.2 ± 0.8	87.2 ± 2.9
Methionine	10.3 ± 0.3	10.0 ± 0.1	10.9 ± 0.5	9.5 ± 0.6
Phenylalanine	37.4 ± 0.4	37.0 ± 1.0	37.4 ± 0.6	37.4 ± 0.6
Threonine	31.2 ± 0.5	30.0 ± 0.0	30.4 ± 1.4	30.8 ± 0.5
Valine	42.4 ± 1.1	41.8 ± 1.1	41.6 ± 1.5	41.3 ± 1.4
Alanine	50.2 ± 1.7	49.9 ± 0.4	50.1 ± 0.6	51.6 ± 1.6
Aspartic acid	85.2 ± 1.9	85.1 ± 1.5	85.7 ± 1.1	86.0 ± 2.1
Cysteine	11.2 ± 2.7	10.7 ± 0.2	10.4 ± 1.0	10.6 ± 1.8
Glutamic acid	122.5 ± 4.6	121.7 ± 0.2	124.8 ± 5.9	124.7 ± 3.4
Glycine	40.0 ± 2.1	40.2 ± 0.5	40.7 ± 1.3	41.1 ± 0.5
Serine	22.4 ± 0.6	22.4 ± 1.6	21.1 ± 0.2	21.9 ± 0.1
Tyrosine	12.2 ± 2.5	11.5 ± 0.3	12.7 ± 3.1	11.2 ± 1.7
Proline	28.3 ± 1.3	28.7 ± 4.1	29.0 ± 1.0	28.3 ± 3.5
Essential amino acids (EAAs)	365.1 ± 7.9	362.5 ± 2.5	368.4 ± 12.8	365.7 ± 1.0
Total amino acids (TAAs)	756.1 ± 13.1	751.2 ± 0.5	760.6 ± 19.2	760.7 ± 4.5

the control ($P < 0.05$). The FSM-20 group showed significantly higher contents of histidine, isoleucine, lysine, valine, alanine, tyrosine, proline, and lower arginine than the control ($P < 0.05$). The FSM-10-FM group showed higher contents of aspartic acid and alanine and lower contents of arginine, isoleucine, cysteine, glycine, and tyrosine than the control ($P < 0.05$), but there was no obvious change found in TFAAs content between FSM-10-FM group and FSM-0 group.

3.5. Serum and Muscle Biochemical Parameters. As shown in Table 8, serum GLU, TP, CHO, and MDA levels in the three FSM groups and serum TG level in FSM-10-SM and FSM-20 groups were lower than those in FSM-0 group ($P < 0.05$). Moreover, serum TAOC and GSH-Px activities in FSM-20 and FSM-10-FM groups, as well as GSH-Px activity in FSM-20 group were also higher than those in FSM-0 group ($P < 0.05$). In muscle biochemical indexes, the FSM-20 and

FSM-10-FM groups showed higher TAOC and SOD activities ($P < 0.05$), while the FSM-20 group had lower LA content than FSM-0 group ($P < 0.05$).

3.6. Nutrient Utilization and Intestinal Digestive Enzyme Activity. As shown in Table 9, the FSM-20 group presented significantly higher activities of protease and amylase in foregut than FSM-0 group ($P < 0.05$). The PER, PR, and LR in FSM-10-SM and FSM-20 groups were numerically increased ($P > 0.05$).

3.7. Intestinal Histology. As shown in Figures 1 and 2, the intestinal VW of FSM-10-SM and FSM-20 groups showed significant increase than FSM-0 group, as the MT in FSM-10-SM group ($P < 0.05$).

3.8. Intestinal Microbial Community. The intestinal microbial diversity is shown in Table 10. The high coverage index of 0.999 for each sample sequence indicated that the

TABLE 7: Free amino acid composition of largemouth bass flesh (wet weight, mg/kg).

Parameters	FSM-0	FSM-10-SM	FSM-20	FSM-10-FM
Arginine	22.5 ± 2.6 ^b	15.9 ± 1.2 ^a	11.8 ± 1.0 ^a	16.5 ± 2.1 ^a
Histidine	678.8 ± 15.5 ^a	762.0 ± 16.6 ^b	827.2 ± 18.3 ^c	683.8 ± 13.0 ^a
Isoleucine	23.2 ± 1.8 ^b	20.4 ± 2.2 ^{ab}	31.0 ± 3.0 ^c	16.6 ± 0.1 ^a
Leucine	55.0 ± 4.2 ^{ab}	53.5 ± 5.0 ^{ab}	59.0 ± 0.1 ^b	46.9 ± 1.1 ^a
Lysine	75.4 ± 0.3 ^a	88.3 ± 1.6 ^b	106.0 ± 4.7 ^c	73.5 ± 0.9 ^a
Methionine	11.8 ± 2.1	11.3 ± 0.8	11.5 ± 0.0	13.4 ± 1.1
Phenylalanine	64.4 ± 1.4	62.1 ± 2.6	63.9 ± 0.4	60.1 ± 1.6
Threonine	61.5 ± 0.5	64.1 ± 1.8	62.7 ± 4.4	55.1 ± 6.3
Valine	31.6 ± 1.9 ^a	31.1 ± 1.4 ^a	37.6 ± 0.6 ^b	29.5 ± 3.1 ^a
Alanine	213.8 ± 2.7 ^a	282.8 ± 6.3 ^b	268.3 ± 30.3 ^b	267.8 ± 3.9 ^b
Aspartic acid	15.0 ± 1.6 ^a	23.1 ± 0.9 ^b	16.0 ± 0.6 ^a	29.6 ± 2.1 ^c
Cysteine	47.9 ± 1.7 ^b	42.9 ± 1.5 ^{ab}	44.9 ± 0.5 ^b	37.9 ± 3.3 ^a
Glutamic acid	92.4 ± 10.1 ^{ab}	103.4 ± 1.3 ^b	97.4 ± 3.4 ^{ab}	85.0 ± 5.5 ^a
Glycine	935.0 ± 80.3 ^b	861.3 ± 43.5 ^{ab}	860.5 ± 15.6 ^{ab}	794.6 ± 1.6 ^a
Serine	61.5 ± 0.5	64.1 ± 1.8	62.7 ± 4.4	55.1 ± 6.3
Tyrosine	47.2 ± 0.9 ^b	50.3 ± 0.1 ^c	54.0 ± 0.9 ^d	40.3 ± 1.7 ^a
Proline	449.2 ± 48.4 ^a	481.4 ± 8.6 ^a	638.8 ± 26.4 ^b	472.3 ± 11.2 ^a
Delicious amino acids (DAAs)	1256.1 ± 69.1	1270.5 ± 50.1	1242.2 ± 18.7	1177.0 ± 5.8
Total free amino acids (TFAAs)	3086.0 ± 15.6 ^{ab}	3198.6 ± 87.8 ^b	3449.8 ± 22.2 ^c	2974.9 ± 15.1 ^a

Note: In the same row, values with the same letter superscripts mean no significant difference ($P > 0.05$), while with different small letter superscripts mean significant difference ($P < 0.05$).

TABLE 8: Serum and muscle biochemical parameters of largemouth bass.

Parameters	FSM-0	FSM-10-SM	FSM-20	FSM-10-FM
Serum				
GLU (mmol/L)	3.40 ± 0.15 ^c	2.94 ± 0.10 ^b	2.20 ± 0.05 ^a	3.05 ± 0.10 ^b
TP (g/L)	239.68 ± 23.97 ^b	171.43 ± 20.76 ^a	159.52 ± 30.30 ^a	161.90 ± 8.25 ^a
TG (mmol/L)	0.84 ± 0.03 ^c	0.68 ± 0.03 ^b	0.58 ± 0.02 ^a	0.79 ± 0.05 ^c
CHO (mmol/L)	2.16 ± 0.05 ^c	0.94 ± 0.08 ^b	0.74 ± 0.08 ^a	1.09 ± 0.13 ^b
TAOC (U/ml)	33.79 ± 9.42 ^a	45.02 ± 2.62 ^{ab}	48.72 ± 5.41 ^b	49.09 ± 3.65 ^b
SOD (U/ml)	105.25 ± 7.82 ^a	114.24 ± 5.24 ^a	141.81 ± 8.78 ^b	111.29 ± 3.57 ^a
GSH-Px (U/ml)	801.03 ± 25.80 ^a	1015.38 ± 30.77 ^c	908.72 ± 27.92 ^b	926.15 ± 11.09 ^b
MDA (nmol/ml)	4.79 ± 0.48 ^b	3.43 ± 0.42 ^a	3.19 ± 0.60 ^a	3.24 ± 0.16 ^a
Muscle				
TAOC (U/mgprot)	1.02 ± 0.11 ^a	1.18 ± 0.27 ^{ab}	1.48 ± 0.16 ^b	1.45 ± 0.19 ^b
SOD (U/mgprot)	158.53 ± 8.34 ^a	171.49 ± 10.82 ^{ab}	195.69 ± 21.15 ^b	196.43 ± 11.35 ^b
GSH-Px (U/mgprot)	226.56 ± 10.30 ^{ab}	208.38 ± 8.25 ^{ab}	204.19 ± 23.90 ^a	237.87 ± 10.17 ^b
Cath-B (ng/gprot)	49.51 ± 2.75	47.95 ± 3.58	46.62 ± 3.61	49.72 ± 0.18
LA (nmol/mgprot)	61.49 ± 6.56 ^b	54.10 ± 6.36 ^{ab}	47.37 ± 0.69 ^a	53.00 ± 4.27 ^{ab}

Note: In the same row, values with the same letter superscripts mean no significant difference ($P > 0.05$), while with different small letter superscripts mean significant difference ($P < 0.05$). GLU, glucose; TP, total protein; TG, triglyceride; CHO, cholesterol; TAOC, total antioxidant capacity; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; Cath-B, cathepsin B; LA, lactate.

TABLE 9: Nutrient utilization and intestinal digestive enzyme activity of largemouth bass.

Parameters	FSM-0	FSM-10-SM	FSM-20	FSM-10-FM
PER	1.65 ± 0.02	1.77 ± 0.17	1.77 ± 0.06	1.63 ± 0.01
PR (%)	28.89 ± 0.29	30.17 ± 2.88	30.44 ± 1.08	28.22 ± 0.11
LR (%)	50.89 ± 0.56	54.45 ± 4.70	55.56 ± 1.68	54.12 ± 0.34
Protease (U/mgprot)	30.98 ± 2.96 ^a	34.77 ± 1.81 ^{ab}	37.18 ± 3.52 ^b	31.08 ± 1.77 ^a
Amylase (U/mgprot)	0.47 ± 0.04 ^a	0.51 ± 0.02 ^{ab}	0.52 ± 0.03 ^b	0.48 ± 0.04 ^{ab}

Note: In the same row, values with the same letter superscripts mean no significant difference ($P > 0.05$), while with different small letter superscripts mean significant difference ($P < 0.05$). PER, protein efficiency ratio; PR, protein retention; LR, lipid retention.

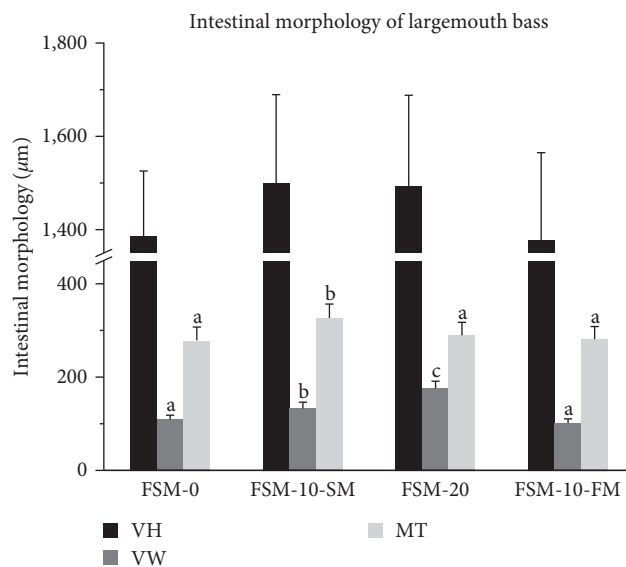


FIGURE 1: Intestinal morphology of largemouth bass. Values are means \pm SD. Bars bearing with different letters are significantly different among treatments ($P < 0.05$). VH, villus height; VW, villus width; MT, muscular thickness.

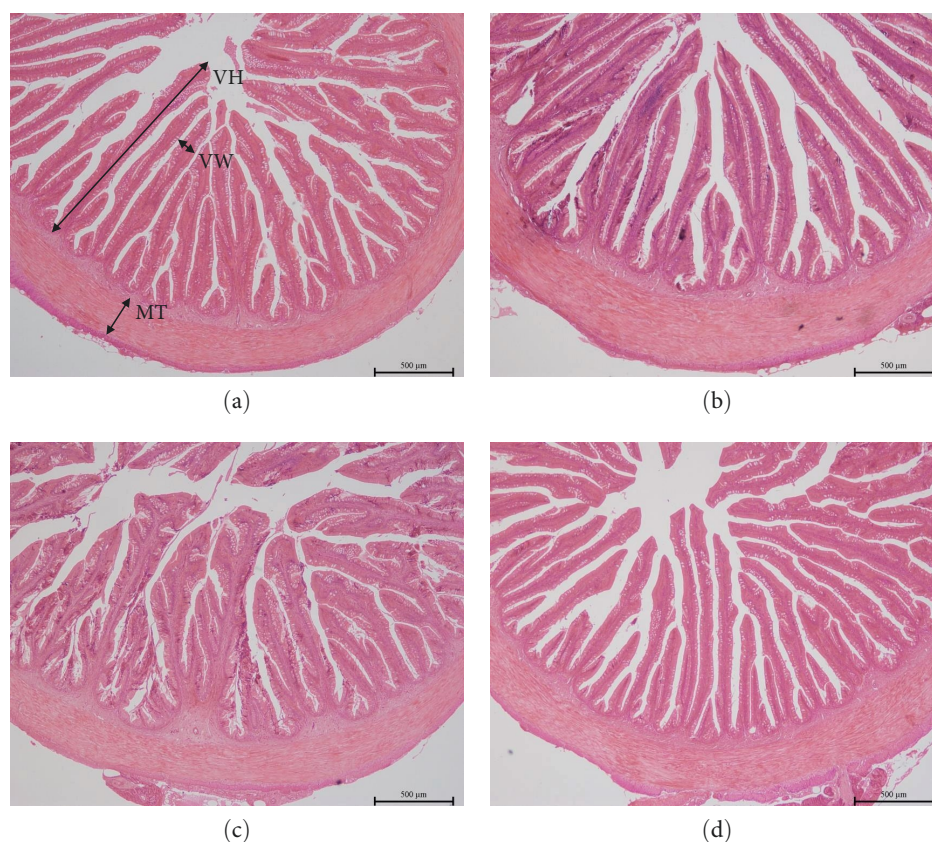


FIGURE 2: Intestinal morphology of largemouth bass (H&E staining, 40x). (a) FSM-0; (b) FSM-10-SM; (c) FSM-20; (d) FSM-10-FM. VH, villus height; VW, villus width; MT, muscular thickness.

TABLE 10: Diversity index on OTU level of intestinal microbial of largemouth bass.

Parameters	FSM-0	FSM-10-SM	FSM-20	FSM-10-FM
Ace	112.9 \pm 35.2	140.8 \pm 7.4	118.5 \pm 39.2	122.1 \pm 29.9
Chao	53.5 \pm 14.1 ^a	137.9 \pm 14.6 ^b	138.7 \pm 20.7 ^b	120.6 \pm 24.1 ^b
Sobs	38.0 \pm 14.1 ^a	130.5 \pm 19.7 ^b	124.5 \pm 38.9 ^b	111.6 \pm 21.2 ^b
Coverage	0.999	0.999	0.999	0.999

Note: In the same row, values with the same letter superscripts mean no significant difference ($P > 0.05$), while with different small letter superscripts mean significant difference ($P < 0.05$).

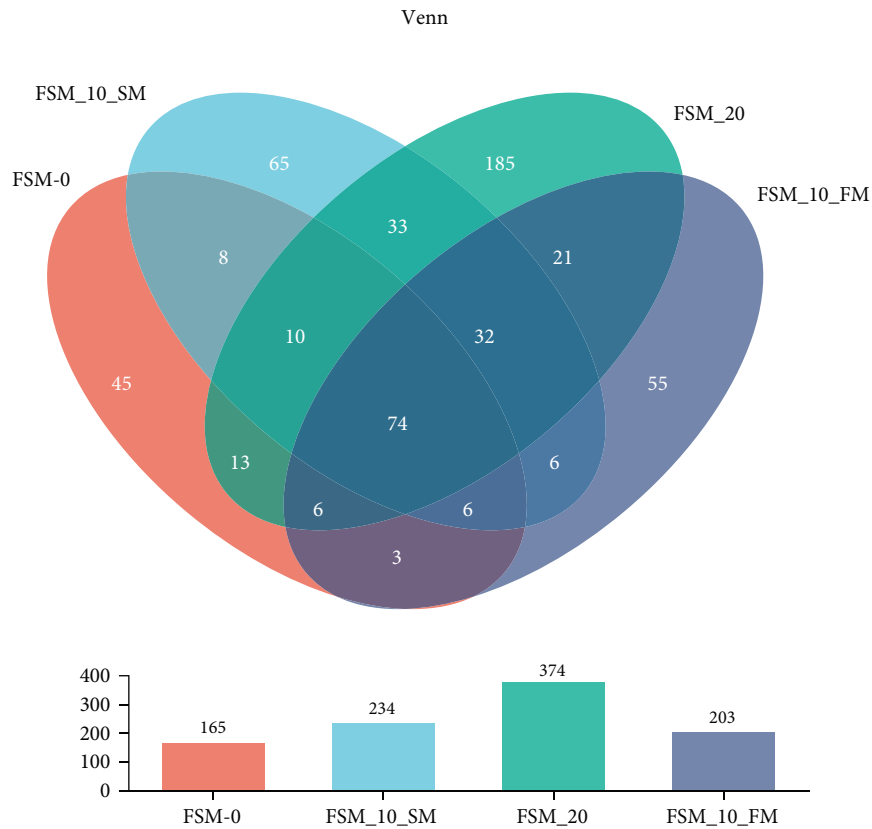


FIGURE 3: Venn diagram of OTUs of largemouth bass. Note: FSM-0 means the control diet without FSM inclusion; FSM_10_SM, FSM_20, and FSM_10_FM represent the FSM diets with 100, 200, and 100 g/kg FSM inclusion to isonitrogenously substitute 120 g/kg soybean meal, 120 g/kg soybean meal + 80 g/kg corn gluten meal, and 65 g/kg fish meal, respectively.

microbial community was sufficiently sampled, and the obtained data were representative of the analyzed samples. The Chao and Sobs indexes of the FSM-10-SM, FSM-20, and FSM-10-FM groups were significantly higher than those of the FSM-0 group ($P < 0.05$). As shown in Figure 3, the Venn diagram of OTUs showed a total of 562 OTUs, with 74 common OTUs found in all groups. There were 165, 234, 374, and 203 OTUs in FSM-0, FSM-10-SM, FSM-20, and FSM-10-FM groups, respectively.

At the phylum level (Figure 4), Firmicutes and Proteobacteria were the main communities in all groups. In the FSM-0, Proteobacteria was found to be the dominant phylum, accounting for 63.96% of the total bacterial population, followed by Firmicutes (20.83%), Fusobacteriota (6.99%), and Verrucomicrobia (6.79%). In the FSM-10-SM, the abundance of Proteobacteria and Fusobacteriota was decreased to 16.56% and 0.11%, while the abundance of Firmicutes was increased to 65.78%. Additionally, the abundance of Actinobacteria and Bacteroidota also showed an increase. In the FSM-20, the abundance of Proteobacteria and Fusobacteriota was observed to decrease, accounting for 15.46% and 1.33%, respectively. Meanwhile, the abundance of Firmicutes, Actinobacteria, and Bacteroidota was increased to 71.62%, 4.60%, and 3.18%. In the FSM-10-FM, the abundance of Proteobacteria and Verrucomicrobia was reduced to 33.86% and 0.01%, and the abundance of Firmicutes was increased to 54.88%.

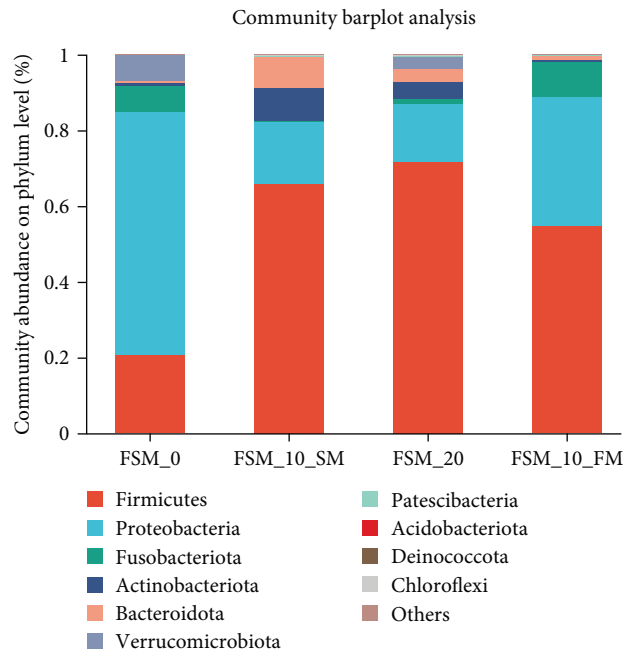


FIGURE 4: Intestinal microbiota abundance of largemouth bass at the phylum level. Note: FSM_0 means the control diet without FSM inclusion; FSM_10_SM, FSM_20, and FSM_10_FM represent the FSM diets with 100, 200, and 100 g/kg FSM inclusion to isonitrogenously substitute 120 g/kg soybean meal, 120 g/kg soybean meal + 80 g/kg corn gluten meal, and 65 g/kg fish meal, respectively.

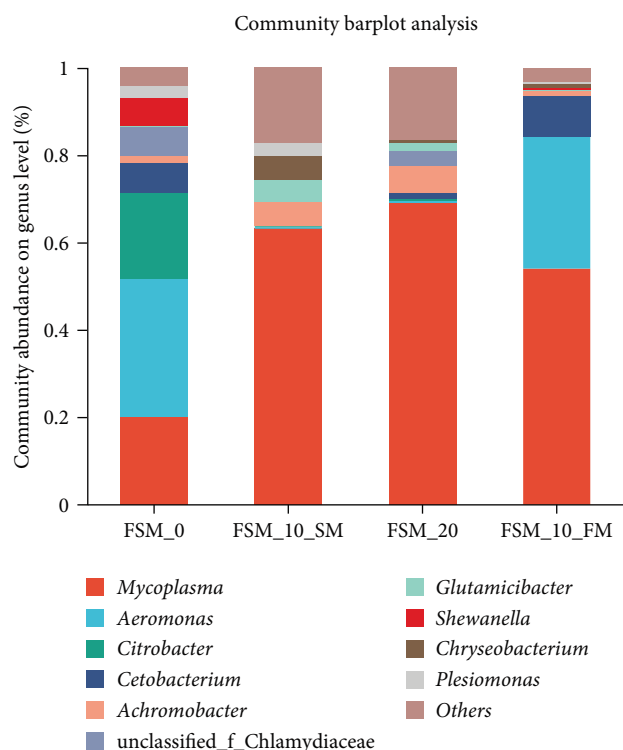


FIGURE 5: Intestinal microbiota abundance of largemouth bass at the genus level. Note: FSM_0 means the control diet without FSM inclusion; FSM_10_SM, FSM_20, and FSM_10_FM represent the FSM diets with 100, 200, and 100 FSM inclusion to isonitrogenously substitute 120 g/kg soybean meal, 120 g/kg soybean meal + 80 g/kg corn gluten meal, and 65 g/kg fish meal, respectively.

The main microorganisms observed at the genus level (Figure 5) were *Mycoplasma* and *Aeromonas*. In the FSM-0, the dominant bacteria were *Aeromonas* (31.42%), *Mycoplasma* (20.11%), and *Citrobacter* (19.85%). In the FSM-10-SM, *Mycoplasma* became the dominant bacteria, accounting for 63.09%, while *Aeromonas* decreased to 0.36%. In the FSM-20, the abundance of *Mycoplasma* and *Cetobacterium* increased to 69.06% and 1.33%, while *Aeromonas* and *Citrobacter* decreased to 0.33% and 0.60%. In the FSM-10-FM, the proportion of *Mycoplasma* increased to 53.96%, and the proportion of *Citrobacter* decreased to 0.09%.

4. Discussion

4.1. Effects of FSM Replacing Plant Protein on Growth Performance. Rombenso et al. [23] found that the FI and WG of hybrid striped bass (white bass *Morone chrysops* × striped bass *M. saxatilis*) were significantly improved by substituting 307 g/kg SM with 303 g/kg FSM in a diet with FM inclusion of 100 g/kg. In turbot, FI, WG, and digestive enzyme activity were also increased by the substitution of 378 g/kg SM with 360 g/kg FSM [12]. In this study, replacing 120 g/kg SM with 100 g/kg FSM tended to enhance the WG, and replacing 120 g/kg SM + 80 g/kg CGM with 200 g/kg FSM greatly enhanced the WG of largemouth bass. Generally, plant proteins contain some antinutritional factors, adversely affecting the palatability and FI [24]. Microbial

fermentation can increase the palatability of feed by degrading saponins, tannins, and other antinutritional factors in plant proteins [25] and produce small peptides, amino acids, and organic acids [6, 10], which may improve the feeding attraction for fish [9]. The enhancement effect of FSM on FI was related to its inclusion level in diets. In this study, FSM-10-SM diet contained 100 g/kg of FSM, and the feed intake-stimulated effect was not significant, while FSM-20 diet with 200 g/kg of FSM inclusion significantly increased the FI (Table 3).

4.2. Effects of FSM Replacing Plant Protein on Flesh Quality. Hardness, springiness, and chewiness are important indicators evaluating flesh quality for consumers. WHC is another significant index of flesh quality. In this study, dietary inclusion of FSM (FSM-10-SM, FSM-20) significantly increased the flesh hardness, shear force of largemouth bass, and tended to increase muscle WHC, which may be correlated with muscle fiber density, collagen content, antioxidant capacity, and pH value. Collagen, the main protein in connective tissue, is positively correlated with muscle fiber density, and higher levels usually contribute to stronger muscle texture [26]. In this study, the total collagen content in muscle of the FSM-20 was increased significantly, and an increasing trend was also observed in the FSM-10-SM, which may be connected with the fact that fermentation enhanced the content of aglycone soybean isoflavones in FSM [27]. Soy isoflavones have been reported to effectively promote the synthesis of skin collagen in human according to Chua et al. [28].

The FAAs content in muscle, especially the flavor amino acids, is an important factor affecting the taste. Flavor amino acids mainly include alanine and glycine, which are sweet, and glutamic acid and aspartic acid, which are umami [29]. In this study, the contents of TFAAs, free alanine, aspartic acid, and glutamic acid in muscle were significantly increased when FSM replaced SM + CGM (FSM-20). The replacement of plant proteins with fermented mixed feed and fermented sorghum distiller's dried grains also significantly increased the flavor amino acids contents in muscle of growing-finishing pig in the studies by Liu et al. [30] and Li et al. [31]. Microbial fermentation can increase the content of FAAs in SM [32], which may contribute to the change of FAAs composition.

4.3. Effects of FSM Replacing Plant Protein on Serum and Muscle Biochemical Indices. In this experiment, the inclusion of 100 and 200 g/kg of FSM significantly increased the TAOC, SOD, and GSH-Px activities and decreased the MDA content of largemouth bass. In previous studies, dietary SM was reported to cause oxidative stress in turbot [11] and rockfish [16]. The β -polyglycine in SM can impair the antioxidant system [33, 34], induce oxidative damage, and cause intestinal digestive and absorption dysfunction in fish [35]. Microbial fermentation of SM not only reduces the β -polyglycine content [18] but also increases the contents of active compounds with antioxidant effects such as soybean active peptides and polyphenols [10]. Uczay et al. [36] used hydrolyzed SM to replace dietary FM and found that the growth performance and antioxidant activity of silver catfish (*Rhamdia quelen*) were improved. Dietary addition of soy isoflavones has also

been reported to improve the growth performance and antioxidant capacity of grass carp (*Ctenopharyngodon idella*) [37].

Gomes et al. [38] once reported that FSM significantly increased the antioxidant enzyme activity in the gastrocnemius muscle of mice. In this study, the TAOC and SOD activities of muscle in FSM-20 were also dramatically improved. Flesh quality is closely related to oxidative damage, and reactive oxygen species can change the protein structure and cleave the peptide chain to form amino acid derivatives, then affect muscle texture characteristics and WHC [39, 40]. Moreover, the higher lactic acid content in muscle will speed up the degradation of myofibrillar protein through calpain system, affecting the texture characteristics and WHC of muscle [26, 41]. Consequently, a proper amount of FSM in diet will contribute to form tight texture, thereby increasing the hardness, shear force, and WHC of muscle.

4.4. Effects of FSM Replacing Plant Protein on Intestinal Histology and Microbiota. High plant proteins diets have been widely documented to trigger intestinal lesions in carnivorous fish [31, 40]. Soyasaponins can induce inflammation in the distal intestine of fish [42]. Soybean lectin can interfere with the digestion and absorption of nutrients in brush border cells by binding epithelial cells in the inner wall of intestinal villi and may lead to intestinal pathological damage [43]. Trypsin inhibitors can affect protein utilization by inhibiting intestinal trypsin activity [1]. In Japanese seabass [44], gibel carp (*Carassius auratus gibelio*) [45], and tilapia (*Oreochromis mossambicus*) [46], the high inclusion of SM in diets decreased the growth performance, digestive enzyme activity, and damaged intestinal tissue structure. The fermentation of SM greatly reduced the contents of antinutritional factors and produced organic acids, active peptides, and other compounds, contributing to the enhancement of intestinal protease and amylase activities in the FSM-20 group (Table 9).

In this study, the replacement of SM and/or CGM by FSM lowered the richness of Proteobacteria and Fusobacteriota and enhanced the richness of Firmicutes, Actinobacteria, and Bacteroidota. In turbot [12], FSM also decreased the richness of Proteobacteria and increased the richness of Firmicutes. The enrichment of Proteobacteria was considered to destabilize intestinal microbial composition and consequently induce disease [47]. Firmicutes and Bacteroidota are usually the main microbiota in healthy intestine. At the genus level, the substitution of plant proteins with FSM improved the richness of *Mycoplasma* and *Cetobacterium* and reduced the richness of *Aeromonas* and *Citrobacter*. He et al. [17] and Zhou et al. [48] found that *Mycoplasma* and *Cetobacterium* were the dominant genera of intestinal bacterial community in largemouth bass, which was also confirmed in the present study. *Aeromonas* is a common opportunistic pathogen that can cause septicemia and gut infection in fish [49, 50]. *Citrobacter freundii*, a representative of *Citrobacter*, has been reported to induce intestinal inflammation and septicemia in freshwater fish such as tilapia [51], catfish [52], and grass carp [53]. Drying is a necessary step in FSM production, and most microorganisms in

FSM would be killed during drying, but some bacterial residues may play beneficial roles similar to probiotics [54]. Besides, the antimicrobial peptides produced by microorganisms during fermentation have an inhibitory and killing effect on pathogenic microorganisms [55]. Thus, the inclusion of FSM positively regulated the intestinal microbial composition in the present study.

4.5. Effects of FSM Replacing FM on Growth Performance. The effect of FSM replacing FM is related to the substitution percentage of FM, fermentation strains, aquatic animal species, and dietary FM content. He et al. [17] reported that *Bacillus subtilis*, *Lactobacillus*, and yeast fermented SM effectively substituted 105 g/kg FM (the basal diet contained 350 g/kg FM) with no effect on the growth performance, but the higher replacement of FM (210 g/kg) significantly increased the FCR and reduced the intestinal VW. In hybrid snakehead (*Channa argus* × *Channa maculata*), the substitution of 50 g/kg FM with lactic acid bacteria fermented SM (the basal diet contained 350 g/kg FM) did not affect the growth performance, but higher replacement reduced FI and WG and negatively affected intestinal protease activity and intestinal tissue structure [56]. In this study, the substitution of 65 g/kg FM by FSM showed no noticeable impact on the growth performance.

4.6. Effects of FSM Replacing FM on Flesh Quality. In this study, the HIS content was significantly reduced in the FSM-10-FM. It is worth noting that the FSM-10-FM showed significantly higher shear force of muscle than the control, indicating that there were some other factors affecting muscle shear force besides the content of HIS, such as the degree of crosslinking of collagen.

In a study by Li et al. [57], replacing 250 g/kg FM with FSM (500 g/kg FM in the basal diet) significantly reduced the content of EAAs in *Nibea diacanthus* muscle. Based on a diet containing 350 g/kg FM, the substitution of 65% of dietary FM with FSM (lysine and methionine were supplemented) did not significantly impact the amino acids content of largemouth bass meat [18]. In this study, the proportion of FSM replacing FM was low (65 g/kg); thus, the amino acid composition and FAAs content in flesh were not significantly affected by the replacement (Tables 6 and 7).

4.7. Effects of FSM Replacing FM on Serum and Muscle Biochemical Indices. In this study, FSM significantly reduced serum GLU and CHO levels. Serum GLU is an important indicator reflecting the body's nutrient intake, and dietary protein and lipid would affect serum GLU levels [58]. The decrease of serum CHO might be associated with the existence of soybean bioactive peptides in FSM [59]. Soybean bioactive peptides can reduce serum CHO content by inhibiting cholesterol biosynthesis and promoting low-density lipoprotein cholesterol metabolism [60]. Soybean bioactive peptide has also been reported to affect low-density lipoprotein receptor activity in the liver of ovariectomized cynomolgus monkeys [61], thereby reducing lipid content in the liver, which probably correlated with a lower VSI in the FSM-10-FM group. The TAOC and GSH-Px activities of the FSM-10-FM group

were higher, while the MDA content was lower than those of FSM-0 group. Duan et al. [56] also observed that replacing FM with FSM significantly improved TAOC and reduced MDA content of hybrid snakehead.

The TAOC and SOD activities in muscle of the FSM-10-FM were also significantly higher than those in the control, aligned with the results of serum antioxidant enzymes. The active substances in FSM may improve flesh quality by promoting the antioxidant enzyme system of fish [62, 63]. However, Liang et al. [14] found that the substitution of 50% FM (180 g/kg) with FSM reduced the flesh hardness and chewiness of Japanese seabass, which may be connected with the excessive decrease in the FM level, thus negatively affecting the synthesis of collagen, although collagen content was not measured in that study.

4.8. Effects of FSM Replacing FM on Intestinal Histology and Microbiota. He et al. [17] reported that replacing 210 g/kg FM with FSM (the basal diet contained 350 g/kg FM) decreased the intestinal VH and VW of largemouth bass. Choi et al. [64] also found that replacing 150 g/kg FM with FSM (250 g/kg FM in the basal diet) reduced the intestinal VH and intestinal protease activity of rainbow trout (*Oncorhynchus mykiss*). In this study, the inclusion level of FSM was 100 g/kg, and the replaced FM was 65 g/kg, which were much lower than the above reports, and no negative effects were detected on intestinal digestive enzyme activity and intestinal histology. It is worth noting that the replacement of FM with FSM increased the richness of Firmicutes and *Mycoplasma* and decreased the richness of Proteobacteria and potential pathogen, *Citrobacter* (Figures 3 and 4). Similarly, the replacement of 115 g/kg FM or 210 g/kg FM with FSM reduced the intestinal number of *Vibrionaceae* in South American catfish [65] and *Escherichia coli* in largemouth bass [17].

5. Conclusion

In this study, based on a 400 g/kg FM inclusion diet, the replacement of 120 g/kg SM or 65 g/kg FM with 100 g/kg FSM did not significantly impact the growth performance but partially enhanced the flesh quality of largemouth bass. The replacement of 120 g/kg SM + 80 g/kg CGM with 200 g/kg of FSM significantly improved the WG and flesh quality such as FAAs content, flesh hardness, and shear force. The FSM inclusion in diets positively regulated the intestinal microbial composition as decreasing the richness of Proteobacteria and increasing the richness of Firmicutes.

Data Availability

All data generated or analyzed during this study are included in this published article.

Conflicts of Interest

The authors have no conflicts of interest with the contents of this article.

Authors' Contributions

Beibei Guo is the first author, and Lingling Huang is the co-first author.

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