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Research Article

Effects of Functional Oligosaccharides Extracted from Straw on the Growth, Feeding, Physiology, Histology, Muscle Texture, and Gut Microbiota of *Micropterus salmoides*

Benli Wu,[1] **Long Huang**,[1] **Cangcang Wu**,[1] **Jing Chen**,[1] **Xiajun Chen**,[1] **Xiang Wang**,[1] **Kai Cui**,[1] **Zhigang Liu**,[2] **and Jixiang He**[1]

[1]*Anhui Province Key Laboratory of Aquaculture & Stock Enhancement, Fisheries Research Institute, Anhui Academy of Agricultural Sciences, Hefei, China 230031*
[2]*CITIC GEYI Recycling Economy Co. Ltd., Huainan, China 232221*

Correspondence should be addressed to Jixiang He; hejixiangah@sina.com

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We conducted an 8-week feeding trial to investigate the effect of mixed functional oligosaccharides (chitosan oligosaccharide, fructooligosaccharide, and xylooligosaccharide) extracted from wheat straw on the growth, feeding, physiology, histology, muscle texture, and gut microbiota of *Micropterus salmoides*. Six diets were formulated by incrementally adding mixed functional oligosaccharides (0.1, 0.5, 1.0, 1.5, and 2.0 mg/kg) to the control diet. 30 individuals with initial body weight of 25.1 ± 3.5 g were randomly allocated to 6 triplicate tanks and fed to apparent satiation twice daily. The fish fed 1.0 mg/kg additives displayed maximum growth, and the feed conversion ratio decreased with the increase in additives from 0 to 1.0 mg/kg ($P < 0.05$) but did not decline further with the addition of more than 1.0 mg/kg ($P > 0.05$). The villus height and width were significantly higher in the supplementation groups than in the control group, while the gut and liver structures presented abnormalities with excessive supplementation above 1.5 mg/kg. There were significant differences in muscle texture indices for *M. salmoides* over the fed additive gradient, and the hardness, gumminess, and chewiness were highest in the 1.0 mg/kg group. High oligosaccharide levels, such as 2.0 mg/kg, decreased the LZM level, while there were no significant differences in the SOD and MDA levels. Gut microbiome analysis revealed no significant differences in richness and diversity for groups fed the functional oligosaccharide gradient; however, the PCoA results showed that the microbial community composition changed markedly in response to different addition levels, and the 0.5 and 1.0 mg/kg supplementation groups were far apart from the lower and higher supplementation groups. The relative abundance of *Proteobacteria* was lower in the 0.5 and 1.0 mg/kg addition groups, while that of the phyla Fusobacteria and Firmicutes were higher in these two groups. Functional classification showed that microbes related to carbohydrate metabolism were more abundant in the 0.5 and 1.5 mg/kg groups than in the other groups.

1. Introduction

The consumption of aquatic products is increasing throughout the world, and aquaculture is rapidly developing to meet the growing demand for high-quality animal protein [1]. However, the animal protein sources for feeds are declining gradually along with the decreased in marine resources so that the quality and stable supply of feeds for aquatic animals has become a challenge for current aquaculture development [2]. Sustainable and valuable substitutes for scarce resources and more effective feed additives thus need to be developed to increase production and reduce the cost of aquatic feeds [3]. Functional feed additives such as probiotics, immunostimulants, organic acids, nucleotides, and various medicinal herb extract are popular in aquafeeds due to their beneficial immunostimulant and physiological

properties, which improve the health and safety of fish and further increase consumer confidence in farmed aquatic products [4].

Functional oligosaccharides are low molecular weight carbohydrates containing 2~10 sugar moieties with different degrees of polymerization, including stachyose [5], raffinose, isomaltulose, isomaltose, gentiooligosaccharide, lactulose, galactooligosaccharide, mannan oligosaccharide [6], fructo-oligosaccharide (FOS), xylooligosaccharide (XOS), and chitosan oligosaccharide (COS) [7–9]. They are not digested and absorbed by the intestines but have multiple physiological activities and the potential to be proliferation factors for probiotics such as *Bifidobacterium*, thus modulating the gut microbiota [10, 11]. Many studies have shown that functional oligosaccharides are potential substitutes for antibiotics such as prebiotics in animal feeds and can enhance the biological and physiological functions of farmed fish and promote the welfare and quality of commercial fish [12–15].

Functional oligosaccharides can be obtained by chemical and enzymatic hydrolysis [16]. Natural COS is primarily derived from crustaceans, insect exoskeletons, and fungal and plant cell walls, while FOS and XOS are mainly extracted from plants, including wheat, rice, and corn [17, 18]. Straw is the largest byproduct of agricultural production, and comprehensive utilization of crop straw is highly valued in many countries to avoid contamination from open burning and develop clean energy [19, 20]. Crop straw has been used for return-to-field or composting as organic fertilizer, sugar [21], ethanol and biogas production [22, 23], power generation [24], straw pulping, artware, daily supplies, and herbivorous animal fodder [25, 26]. Untreated straw cannot meet the requirements of animal production due to its low crude protein content and digestibility and poor palatability [27]. New approaches to the integrated process for straw utilization have developed with the progress of industry and biotechnology [28, 29]. Among these, wheat straw has gained increasing interest as a biorefinery feedstock due to its wide distribution. It contains 35–45% cellulose, 20–30% hemicelluloses, and 15–20% lignin, making this biomass an attractive raw material for conversion to ethanol and other value-added products such as functional oligosaccharides [30].

China is a large agricultural country, and substantial amounts of agricultural byproducts, such as corn cob, cotton seed hull, and straw, that are rich in cellulose-type xylanase are produced every year [31]. Extraction of oligosaccharides from natural sources may also lead to the discovery of novel prebiotics [32]. Utilization of low-cost agroresidues as substrates for producing oligosaccharides has become an economical approach [33]. For example, rice straw, corn straw, corncob, and wheat straw can serve as raw materials for the production of oligosaccharides within the biorefinery framework along with the production of biofuels [34, 35]. In addition to being used as feed additives, oligosaccharides can also be used as fertilizer and plant disease vaccines [36]. At present, functional oligosaccharides are extracted from crop straw, mainly wheat straw [37]. The total area planted with wheat in China was 2.36×10^7 hectares, and the production of wheat in 2021 reached 1.37×10^8 t, which meant that approximately 1.56×10^8 t of wheat straw was produced in 2021 [38, 39]. The utilization of straw extraction products as feed additives is conducive to the healthy development of the feed industry; moreover, the clean utilization of straw contributes to environmental protection [40].

Largemouth bass (*Micropterus salmoides*) has been an important freshwater farmed fish in recent years [41–43]. Intensive aquaculture and formulated feed intake can cause fatty liver and enteritis. The present study evaluated the effects of functional oligosaccharides extracted from wheat straw on the growth, feeding, physiology, histology, muscle texture, and gut microbiota of *M. salmoides*. It is expected that the study will provide evidence for feed optimization and development of another clean utilization method for straw.

2. Materials and Methods

2.1. Fish Rearing. The fingerlings of *M. salmoides* used in this experiment were obtained from a commercial farm (Huoshan, Anhui). Fish were acclimatized in an indoor recirculating aquaculture system with basal feed for two weeks. Then, 30 individuals with an initial body weight of 25.1 ± 3.5 g were randomly allocated to 6 triplicate tanks (200 L) for the feeding trial. Fish were fed to apparent satiation with two meals per day at 09 : 00 and 16 : 00 during the 8 experimental weeks. The feed intake was monitored at each meal. Water was slightly aerated to ensure sufficient dissolved oxygen (DO). The DO was maintained at 6.29–6.78 mg/L during the trial, and the ambient water temperature varied from 25.8 to 16.4°C and pH from 7.5 to 7.8. The photoperiod was 12 h light : 12 h dark. During the feeding trial, still water culture without recycled or new water was adopted during two 7-day periods (15–21 d and 43–49 d) to monitor the variation in pH, ammonia-nitrogen, and nitrite.

2.2. Experimental Diets. The functional oligosaccharides extracted from straw contained 24.5% chitosan oligosaccharide (COS), 4.1% xylooligosaccharide (XOS), and 5.2% fructooligosaccharide (FOS). To determine the effective supplementation level, we conducted a short-term (4-week) preliminary feeding experiment for juvenile largemouth bass at five oligosaccharide concentrations (0, 0.1, 1, 10, 100, and 1000 mg/kg). Growth and feed efficiency were observed, and the addition of 1 mg/kg resulted in better growth performance. Based on the results, experimental diets were supplemented with gradient levels of functional oligosaccharides at 0, 0.1, 0.5, 1.0, 1.5, and 2.0 mg/kg, termed C0, C1, C2, C3, C4, and C5, respectively (Table 1). The particle size of the expanded feeds was 2.0 mm.

2.3. Measurement and Sampling. Fish were group-weighed at 28 d and 56 d and individually measured at the end of the trial to obtain the growth index. Measuring and sampling were performed after the fish were fasted for 24 h and anesthetized (150 mg/L MS 222, Sigma, USA). Three fish in each tank were randomly selected to draw 2.0 mL plasma from

Table 1: Ingredients and chemical composition of experimental diets with gradient supplementation levels of functional oligosaccharides (%, as fed basis).

Item	Diet					
	C0	C1	C2	C3	C4	C5
Ingredients						
Fish meal	25	25	25	25	25	25
Shrimp meal	5	5	5	5	5	5
Fermented soybean meal	6	6	6	6	6	6
Soybean meal	15	15	15	15	15	15
Peanut bran	4	4	4	4	4	4
Beer yeast	3	3	3	3	3	3
Wheat flour	18	18	18	18	18	18
Wheat middlings	5	5	5	5	5	5
Soybean phosphate	1.5	1.5	1.5	1.5	1.5	1.5
Soybean oil	0.75	0.75	0.75	0.75	0.75	0.75
Fish oil	0.75	0.75	0.75	0.75	0.75	0.75
Vitamin premix[1]	0.5	0.5	0.5	0.5	0.5	0.5
Mineral premix[2]	1	1	1	1	1	1
Microcrystalline cellulose	1	0.999	0.995	0.99	0.985	0.98
Functional oligosaccharides[3]	0	0.001	0.005	0.01	0.015	0.02
Chemical composition (%)						
Crude protein	44.8	44.9	44.8	45.0	44.9	45.0
Crude lipid	10.5	10.4	10.4	10.3	10.5	10.4
Ash	8.8	8.8	8.9	8.8	8.9	8.9
Gross energy (kJ/100 g)	1824	1813	1825	1816	1823	1817

[1]Vitamin premix (per kilogram diet): vitamin A, 20 mg; vitamin D_3, 10 mg; vitamin E, 200 mg; vitamin K_3, 10 mg; vitamin B_1, 15 mg; vitamin B_2, 15 mg; vitamin B_6, 15 mg; vitamin B_{12}, 0.08 mg; D-biotin, 0.8 mg; D-calcium pantothenate, 40 mg; folic acid, 5 mg; niacinamide, 50 mg; vitamin C, 140 mg; and inositol, 120 mg. [2]Mineral premix (per kilogram diet): $FeSO_4$, 40 mg; $CuSO_4.5H_2O$, 15 mg; $MnSO_4.4H_2O$, 100 mg; $ZnSO_4$, 150 mg; $MgSO_4.7H_2O$, 200 mg; $CoCl_2.6H_2O$, 5 mg; KI, 1 mg; and Na_2SeO_3, 5 mg. [3]Functional oligosaccharides contained chitosan oligosaccharide (24.5%), fructooligosaccharide (5.2%), and xylooligosaccharide (4.1%).

the caudal vein using syringes (3.0 mL), and the plasma was centrifuged (4000 r/min, 8 min) to separate the serum and kept at -20°C before further analysis. Another six individuals with similar body sizes from each tank were sacrificed to measure visceral coefficients and muscle texture indices. The viscera were stripped, and then, the liver, gut, and abdominal fat were separated and weighed. The liver and gut from three individuals were quickly frozen in liquid nitrogen and then stored at -80°C for physiological analysis, and the other livers and guts (proximal intestine) were fixed with 4% paraformaldehyde for histological analysis. Dorsal muscles in the same location were cut and trimmed into small cubes (1 cm × 1 cm × 0.5 cm) for texture analysis. Three guts from each group were carefully removed on ice under sterile conditions for microbial community analysis. All operations on fish were conducted in accordance with the institutional animal care guidelines and under the supervision of the Experimental Animal Welfare and Ethics Committee of Anhui Academy of Agricultural Sciences (No. AAAS 2020-21).

The parameters measured and the corresponding calculations were follows:

$$\begin{aligned}
\text{Specific growth rate (SGR, \%/d)} &= 100 \times \frac{(\ln \text{BW} - \ln \text{BW}_0)}{T},\\
\text{Condition factor } \left(\text{CF, g/cm}^3\right) &= 100 \times \frac{\text{BW}}{\text{BL}^3},\\
\text{Feed conversion ratio (FCR)} &= \frac{\text{FI}}{(\text{BW} - \text{BW}_0)},\\
\text{Visceral-somatic index (VSI, \%)} &= 100 \times \frac{\text{visceral mass weight}}{\text{BW}},\\
\text{Hepato-somatic index (HSI, \%)} &= 100 \times \frac{\text{liver weight}}{\text{BW}},\\
\text{Intestine somatic index (ISI, \%)} &= 100 \times \frac{\text{intestine weight}}{\text{BW}},\\
\text{Intestinal coefficient (IC)} &= \frac{\text{intestine length}}{\text{BL}},\\
\text{Mesenteric fat index (MFI, \%)} &= 100 \times \frac{\text{mesenteric fat weight}}{\text{BW}},
\end{aligned} \tag{1}$$

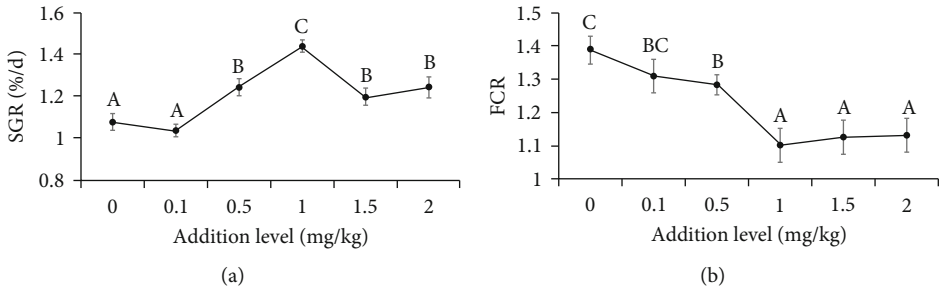

Figure 1: Specific growth rate (SGR) (a) and feed conversion ratio (FCR) (b) for *M. salmoides* fed diets with a gradient level of functional oligosaccharides. Different superscript letters indicate significant differences ($P < 0.05$).

Table 2: Body weights and organ coefficients of *M. salmoides* fed diets with increasing levels of functional oligosaccharides.

Groups	28 d BW (g)	56 d BW (g)	CF (g/cm^3)	VSI (%)	HSI (%)	ISI (%)	IC	MSI (%)
C0	40.22 ± 2.88^{ab}	45.84 ± 4.86^{a}	1.968 ± 0.059	7.26 ± 0.37	1.40 ± 0.28^{a}	0.66 ± 0.13	0.80 ± 0.05	1.26 ± 0.12^{a}
C1	40.55 ± 3.56^{ab}	44.83 ± 4.66^{a}	1.955 ± 0.030	7.16 ± 0.40	1.63 ± 0.26^{ab}	0.64 ± 0.17	0.80 ± 0.05	1.29 ± 0.21^{a}
C2	42.52 ± 2.33^{b}	50.31 ± 3.10^{ab}	1.899 ± 0.023	7.54 ± 0.32	1.83 ± 0.25^{b}	0.60 ± 0.13	0.80 ± 0.08	1.43 ± 0.19^{ab}
C3	41.89 ± 3.12^{b}	56.17 ± 3.48^{b}	1.952 ± 0.009	7.37 ± 0.21	1.94 ± 0.22^{b}	0.58 ± 0.14	0.79 ± 0.09	1.55 ± 0.16^{b}
C4	38.72 ± 1.89^{a}	49.05 ± 4.24^{ab}	1.929 ± 0.051	7.55 ± 0.25	1.89 ± 0.21^{b}	0.62 ± 0.13	0.83 ± 0.06	1.45 ± 0.12^{ab}
C5	39.67 ± 2.78^{ab}	50.31 ± 3.61^{ab}	1.932 ± 0.021	7.48 ± 0.54	1.83 ± 0.29^{b}	0.63 ± 0.13	0.80 ± 0.04	1.43 ± 0.11^{ab}

The different superscript letters indicate significant differences (P<0.05).

where BW_0, T, and FI are the initial body weight, feeding day, and feed intake, respectively. BW and BL are body weight and body length, respectively.

2.4. Physiological, Biochemical, and Histological Analyses. Total protein (TP), cholesterol (CHO), triglyceride (TG), lysozyme (LZM), superoxide dismutase (SOD), and malonaldehyde (MDA) in the serum and liver were determined using commercial kits (Jiancheng Biotech. Co., Nanjing, China) and spectrophotometric methods (UV 2800, Shimadzu) following the manufacturer's specifications. Fixed liver and gut tissues were made into paraffin slices and observed after hematoxylin-eosin (HE) staining under a microscope (CX43, Olympus). The muscle texture indices hardness, springiness, resilience, cohesiveness, gumminess, and chewiness were tested using a texture analyzer with a TPA36 cylindrical probe (TA.XT Plus, SMS). The deformation was set at 40%, the testing speed was 2 mm/s, the distance was 5 mm, the second distance was 10 mm, and the trigger force was 5.0 g.

2.5. Gut Microbiota Analysis. Microbial community DNA was extracted from gut samples using the TIANamp Stool DNA Kit (DP328, TIANGEN, Beijing, China) according to the manufacturer's instructions (E.Z.N.A.® soil DNA Kit, Omega Bio-tek, Norcross, GA, USA). The DNA extract was checked on a 1% agarose gel, and the DNA concentration and purity were determined with a NanoDrop 2000 UV–Vis spectrophotometer (Thermo Scientific, Wilmington, USA). The V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified with primer pairs 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). The PCR amplification of the 16S rRNA gene was performed as follows: initial denaturation at 95°C for 3 min; 29 cycles of denaturing at 95°C for 30 s, annealing at 53°C for 30 s, and extension at 72°C for 45 s; a single extension at 72°C for 10 min; and a final extension at 4°C. The PCR mixtures contained 4 μL 5x TransStart FastPfu buffer, 2 μL 2.5 mM dNTPs, 0.8 μL forward primer (5 μM), 0.8 μL reverse primer (5 μM), 0.4 μL TransStart FastPfu DNA Polymerase, 10 ng template DNA, and ddH_2O up to 20 μL. PCRs were performed in triplicate. The PCR product was run on a 2% agarose gel, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions, and quantified using a Quantus™ Fluorometer (Promega, USA). Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina MiSeq PE300 platform (Illumina, San Diego, USA) according to standard protocols by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China).

The raw 16S rRNA gene sequencing reads were demultiplexed, quality-filtered by fastp version 0.20.0, and merged by FLASH version 1.2.7. Operational taxonomic units (OTUs) with a 97% similarity cutoff were clustered using UPARSE version 7.1, and chimeric sequences were identified and removed. The taxonomy of each OTU representative sequence was analyzed by RDP Classifier version 2.2 against the 16S rRNA database (SILVA v138) using a confidence

Table 3: Serum and liver biochemical parameters of *M. salmoides* fed diets with increasing levels of functional oligosaccharides.

Groups	Serum			Liver		
	TP (gprot/L)	TG (mmol/L)	CHO (mmol/L)	SOD (U/mgprot)	MDA (nmol/mgprot)	LZM (μg/mL)
C0	20.25 ± 0.45^{a}	4.10 ± 0.11^{a}	17.81 ± 1.51^{c}	205.88 ± 16.33	7.42 ± 0.35	27.32 ± 2.33^{b}
C1	20.25 ± 0.38^{a}	3.82 ± 0.16^{a}	15.38 ± 1.46^{ab}	196.82 ± 17.96	6.77 ± 0.42	28.57 ± 2.54^{b}
C2	20.40 ± 0.23^{a}	4.41 ± 0.15^{b}	14.40 ± 1.19^{a}	208.22 ± 20.21	6.92 ± 0.40	27.38 ± 1.96^{b}
C3	22.21 ± 0.25^{b}	5.58 ± 0.12^{d}	17.40 ± 2.00^{c}	223.94 ± 15.46	7.15 ± 0.39	26.04 ± 2.36^{b}
C4	19.80 ± 0.16^{a}	4.98 ± 0.11^{c}	16.49 ± 1.34^{b}	209.02 ± 18.22	7.20 ± 0.38	25.35 ± 1.89^{b}
C5	19.95 ± 0.13^{a}	4.40 ± 0.13^{b}	15.25 ± 1.54^{ab}	210.87 ± 17.93	6.92 ± 0.23	23.04 ± 1.11^{a}

The different superscript letters indicate significant differences ($P<0.05$).

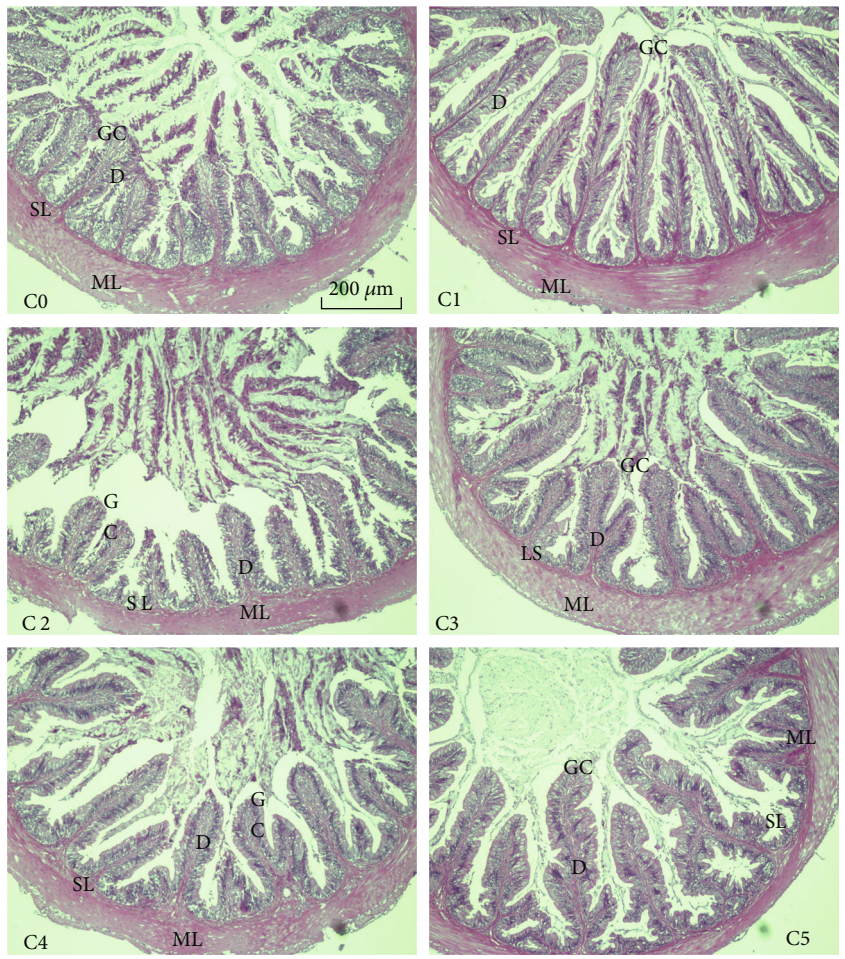

Figure 2: Gut morphology of *M. salmoides* fed diets with a gradient level of functional oligosaccharides. ML: muscularis; SM: submucosa; D: duplicature; GC: goblet cell.

threshold of 0.7. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (accession number: PRJNA728850) (http://trace.ncbi.nlm.nih.gov/Traces/sra/).

2.6. Statistical Analysis. Data analysis and visualization were conducted in SPSS 19.0 (Chicago, IL, USA) and Microsoft Excel 2010. Homoscedasticity was tested for biometric measurements, and then, one-way analysis of variance (ANOVA) was conducted when $P > 0.05$. Duncan's multiple comparisons were conducted to assess the effect of different supplementation levels of functional oligosaccharides, where $P < 0.05$ indicated a significant difference. The data are presented as mean ± SD. All statistical analyses of the gut microbiota were conducted on a cloud platform (http://www.majorbio.com).

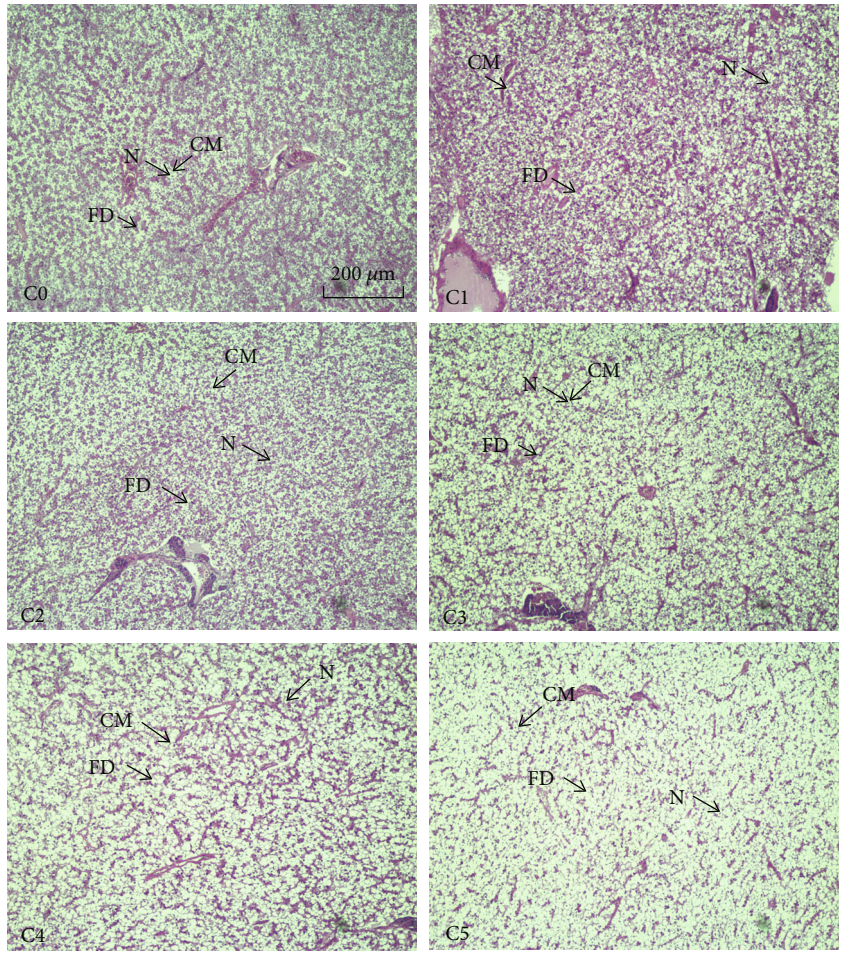

Figure 3: Liver morphology of *M. salmoides* fed diets with a gradient level of functional oligosaccharides. FD: fat droplet; N: nucleus; CM: cell membrane.

3. Results

3.1. Growth Performance and Feed Intake. There was no mortality during the feeding trial for all groups. The maximum body weight occurred in C2 at 28 d and C3 at 56 d. C3 had the highest SGR in the 8-week feeding trial, and the final body weights of C0 and C1 were significantly lower than those of C2–C5 ($P < 0.05$) at 56 d (Figure 1(a)). There was no significant difference among the groups in CF, VSI, IC or ISI. However, the HSI and MFI were significantly lower in the groups with low supplement diets, such as C0 and C1 (Table 2). The FCR decreased with the increase in functional oligosaccharides from 0 to 1.0 mg/kg but did not decline further with the addition of more than 1.0 mg/kg (Figure 1(b)). Overall, the C3 group presented optimal growth and feed efficiency during the feeding trial.

3.2. Biochemical and Immune Indicators. The serum TP, TG, and CHO were significantly higher in C3 than in the other experimental groups ($P < 0.05$), but there was no significant difference between C0 and C3 in CHO ($P > 0.05$). SOD was relatively higher in C3, but the difference was not significant among groups ($P > 0.05$). MDA levels were relatively lower in the treatment groups than in the C0 group. The LZM level in C5 was significantly lower than that in C0–C4 ($P < 0.05$) (Table 3).

3.3. Histology of the Liver and Gut. Analysis of the gut morphology showed that the villus height and width were significantly higher in C1–C5 than in C0. The villus was more intact in C1, C2, and C3 but damaged in C5. The muscularis (ML) thickness was higher in the low oligosaccharide groups than in the high oligosaccharide groups (Figure 2). For the liver, fat droplets (FD) were larger and more widespread in the high oligosaccharide groups. Nuclear deviation, vacuolar degeneration, fat accumulation, and unclear cell contours were ubiquitous in C4 and C5 (Figure 3).

3.4. Muscle Texture. There were significant differences in muscle texture indices for *M. salmoides* fed a gradient of functional oligosaccharides. The hardness was lower in C0, C1, and C5 than in C2–C4 ($P < 0.05$). Springiness was significantly lower in C0 and C1 than in C4 ($P < 0.05$), and gumminess was higher in C3 and C4 than in C0, C1, and C5 ($P < 0.05$). The chewiness was higher in C3 and C4 than in C0 ($P < 0.05$). There was no significant difference in cohesiveness among the groups ($P > 0.05$) (Table 4).

Table 4: Muscle texture of *M. salmoides* fed diets with a gradient of functional oligosaccharides.

Groups	Force	Hardness	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
C0	1143.8 ± 67.5^{a}	1421.9 ± 85.0^{a}	0.55 ± 0.01^{a}	0.49 ± 0.01	705.1 ± 45.1^{a}	397.5 ± 30.5^{a}	0.30 ± 0.01^{ab}
C1	1215.4 ± 84.0^{ab}	1503.8 ± 100.8^{ab}	0.55 ± 0.01^{a}	0.48 ± 0.01	748.8 ± 58.2^{ab}	432.8 ± 39.1^{ab}	0.29 ± 0.01^{a}
C2	1364.3 ± 66.5^{b}	1668.7 ± 76.2^{b}	0.58 ± 0.01^{ab}	0.50 ± 0.01	848.9 ± 51.4^{abc}	508.0 ± 37.4^{abc}	0.31 ± 0.01^{ab}
C3	1534.7 ± 81.3^{c}	1872.1 ± 95.4^{c}	0.58 ± 0.01^{ab}	0.49 ± 0.01	933.8 ± 59.4^{c}	554.3 ± 42.2^{c}	0.30 ± 0.01^{ab}
C4	1400.2 ± 81.1^{bc}	1734.4 ± 98.4^{bc}	0.59 ± 0.01^{b}	0.51 ± 0.01	891.8 ± 55.4^{bc}	543.4 ± 40.9^{bc}	0.33 ± 0.01^{b}
C5	1215.0 ± 80.7^{ab}	1510.0 ± 99.6^{ab}	0.58 ± 0.01^{ab}	0.47 ± 0.01	753.1 ± 56.4^{ab}	452.1 ± 40.5^{ab}	0.31 ± 0.01^{ab}

The different superscript letters indicate significant differences ($P<0.05$).

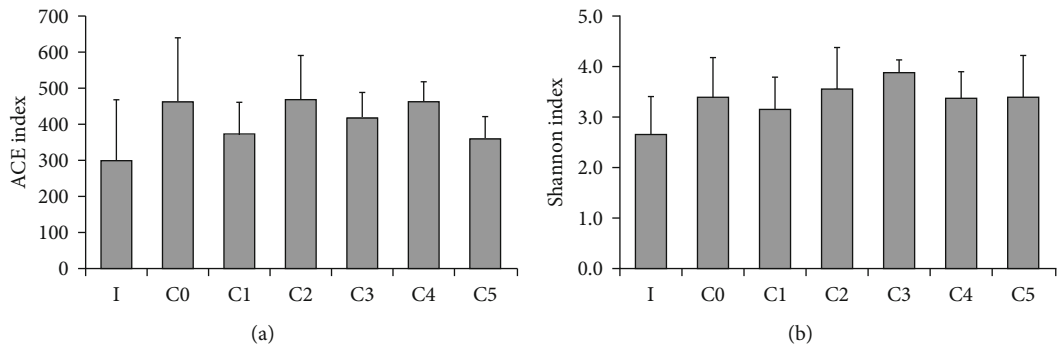

Figure 4: The ACE (a) and Shannon (b) indices for initial fish and groups fed diets with gradient levels of functional oligosaccharides.

3.5. Gut Microbiota. A total of 1,195,409 valid bacterial 16S rRNA gene reads were obtained, which varied from 47,648 to 70,236 for all samples. A total of 1,536 OTUs were identified from all samples, and the OTU richness varied from 137 to 587 among the samples. Rarefied OTUs (with reads normalized to 4,7648 for each sample) were adopted for diversity and richness analyses. We chose the ACE and Shannon indices as richness and evenness indicators, respectively. Both the ACE and Shannon indices were lower in the initial group (I), and there were no significant differences in the ACE or Shannon indices for groups fed a gradient of functional oligosaccharides (Figure 4). The results of principal coordinate analysis (PCoA) based on the Bray–Curtis distances showed clear distinctions between initial fish and fish after the feeding trial. Furthermore, the microbial community composition changed markedly in response to different addition levels of functional oligosaccharides, and groups C2 and C3 were far apart from the lower and higher supplement groups. However, there were no clear distinctions among C0, C1, C4, and C5; in addition, groups C2 and C3 were relatively close (Figure 5).

3.5.1. Dominant Microbes. For all samples, the recognized microbes belonged to 26 phyla, 62 classes, 163 orders, 283 families, 569 genera, and 1,536 OTUs (956 species were recognized) from all the samples based on SILVA v138. The phylum and genus levels were emphasized in the analysis. Proteobacteria, Firmicutes, Bacteroidetes, Fusobacteria, and Actinobacteriota were the most dominant phyla, accounting for more than 95% of the total bacteria in all groups. Fuso-

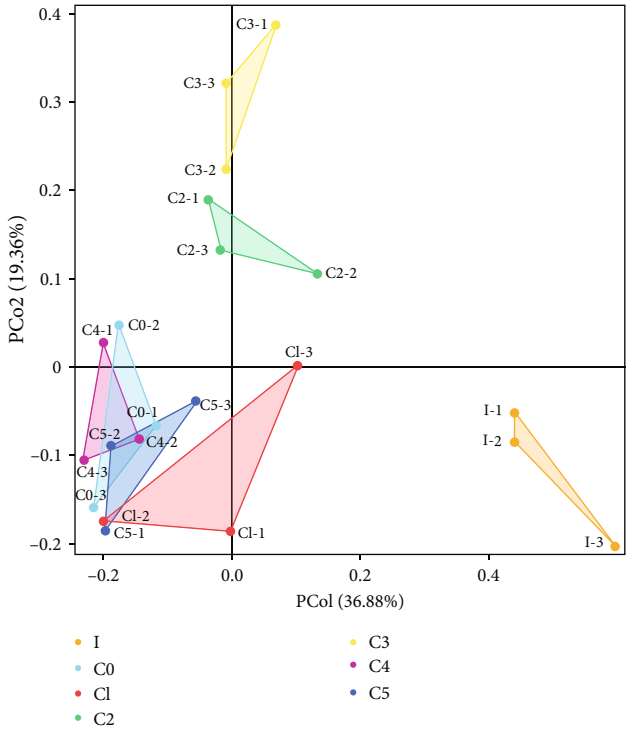

Figure 5: Principal coordinate analysis (PCoA) based on the Bray–Curtis distances. Different symbols represent different groups fed diets with a gradient level of functional oligosaccharides.

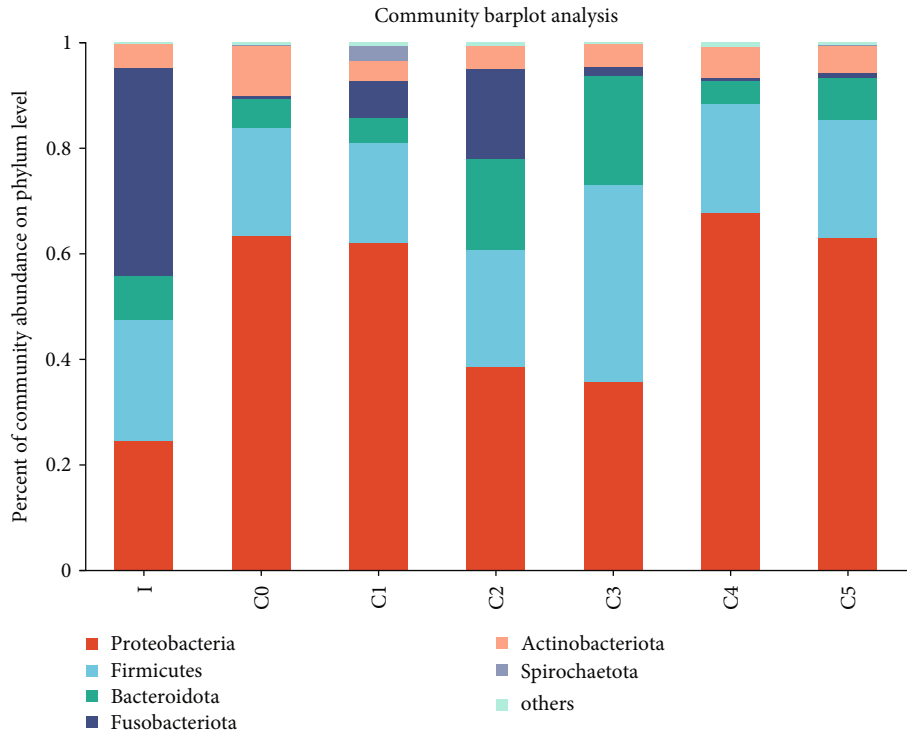

Figure 6: Dominant phyla in the gut microbial community of different groups fed a gradient level of functional oligosaccharides.

bacteria was the most abundant phylum in the initial fish, while Proteobacteria was the most abundant phylum after the fish were fed diets with a gradient of functional oligosaccharides. When compared to the C0 group, the relative abundance of Proteobacteria was lower in C1, C4, and C5, while the relative abundance of Fusobacteria and Firmicutes was higher in the C2 and C3 groups (Figure 6).

There was a significant difference in the dominant genera among the initial samples and subsequent samples after the feeding trial. The most dominant genus in the initial samples was *Cetobacterium*, while it was *Bosea* for the groups after the feeding trial. However, the relative abundance of *Bosea* was lower in C3, and *Cetobacterium* was more abundant in C3 than in the other groups (Figure 7). There were 272 common species among the experimental groups. The number of specific microbes was highest in C1 and lowest in C5 (Figure 8).

LEfSe analysis was also conducted to identify representative microbes among various groups. For the initial fish, representative microbes belonged to *Haematospirillum*, *Snodgrassella*, *Acidaminococcus*, *Geobacillus*, Paenibacillus, Rhodospirillaceae, and Rhodospirillales. Among the groups fed a gradient of oligosaccharide addition, the C3 group contained more representative species, including members of Clostridia-UCG-104, *Ruminococcaceae*, *Sellimonas*, *CHKCI001*, and *Colidextribacter*, which presented higher LDA scores than those of the other groups. The C5 group contained few representative microbes, such as *Subdoligranulum* (Figure 9).

3.5.2. Functional Predictions. In total, 47 predicted functional categories that represented 7 pathway maps in level 2 were indicated by PICRUSt, including 369 functions on level 3. The functional classification showed that supplementation with functional oligosaccharides had significant effects on energy, carbohydrate, lipid, and amino acid metabolism. The microbes related to carbohydrate metabolism, such as fructose and mannose metabolism, galactose metabolism, starch, and sucrose metabolism, were more abundant in C2 and C3 than in C0, C1, C4, and C5, while the relative abundance of microbes related to fatty acid metabolism was lower in C2 and C3 (Figure 10).

3.6. Variation in Culture Water. The NH_4^+-N content increased gradually in the control group and low oligosaccharide addition groups such as C1, while for the high oligosaccharide addition groups such as C3, C4, and C5, it increased at 0–5 d but declined at 5–7 d, and the content was <0.5 mg/L at 7 d when the added oligosaccharide level was >1.0 mg/kg. The nitrite content increased gradually during the 7 d feeding. The accumulation speed was considerably lower in the high oligosaccharide addition groups. After 7 days of feeding, both the NH_4^+-N and nitrite contents were significantly higher in the control group and low oligosaccharide addition groups than in the high oligosaccharide addition groups ($P < 0.05$, Figure 11).

4. Discussion

Functional oligosaccharides are a new type of green feed additive. Many studies on different species have shown their positive effects on growth, immunity, disease resistance, antimicrobial and antioxidant activity, and immune-related gene expression [44, 45]. Abdel-Ghany and Salem summarized the effects of dietary chitosan on farmed aquatic

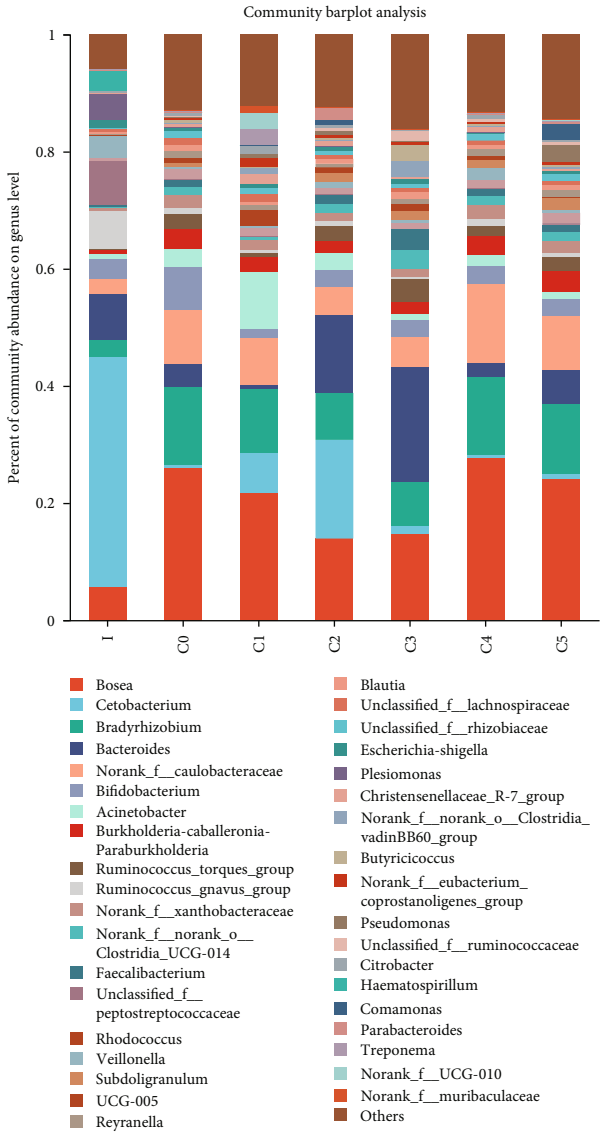

Figure 7: Dominant genera in the gut microbial community of different groups fed a gradient level of functional oligosaccharides.

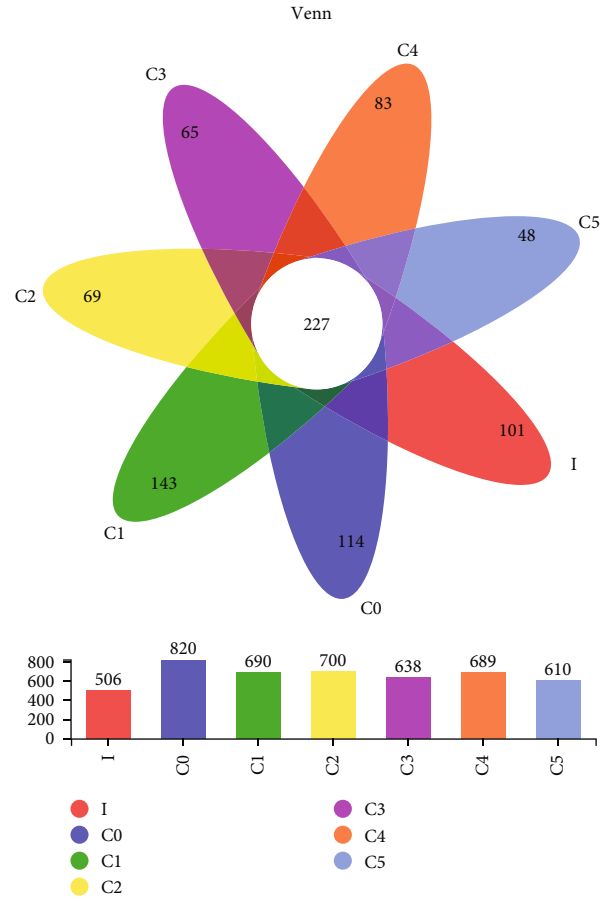

Figure 8: Common and unique OTUs in different groups fed a gradient level of functional oligosaccharides.

animals and concluded that the recommended concentration ranged from 0.5 to 500 mg/kg for various species, body sizes, and culture environments [13]. Functional oligosaccharides are a type of functional carbohydrate that cannot be easily absorbed by the gut but can improve intestinal health with proper addition. The excessive addition of functional oligosaccharides also showed adverse effects on the growth, physiological activities, and structure of the liver and gut [46, 47]. The additives promoted growth and feed efficiency, and the HSI was significantly higher in the fast-growing groups (C2–C5) than in the relatively slow-growing group (C0) in this study. However, the histomorphological observation of the liver showed abnormalities in the high addition groups, especially in C4 and C5, with more or larger fat droplets and irregular cells, which also indicated higher metabolic pressure in these groups. The purification of oligosaccharides from straw should be further optimized to eliminate potential toxins, and the addition level and proportion of different components should also be further studied to reduce side effects.

There was no significant difference in antioxidant capacities involving the activities of SOD and MDA among all groups with oligosaccharide addition, but the LZM decreased with increasing addition of oligosaccharides in the present study. Liu et al. indicated that high dietary carbohydrate content significantly reduced LZM content but increased complement C4 content in largemouth bass [48]. The underlying immune regulation mechanisms are not well characterized, but high oligosaccharide addition imposes a certain burden on the liver and intestine and further affects immunity indicators. However, this might be due not only to the excessive additives but also to the increased feed intake of diets during the feeding trial. Therefore, the addition level should also consider the existing oligosaccharides in feed ingredients such as shrimp meal, soybean, and wheat.

The texture of fish muscle is an important freshness quality attribute. Physical factors affecting muscle texture include age, body size, seasonal changes, feeding ingredients, sample heterogeneity, and gaping [49]. The texture indices were higher in individuals with larger body sizes in this study, and the feeding ingredients were deemed to be the main factor affecting texture indices. Oligosaccharide supplementation could help restore muscle texture properties by regulating the expression

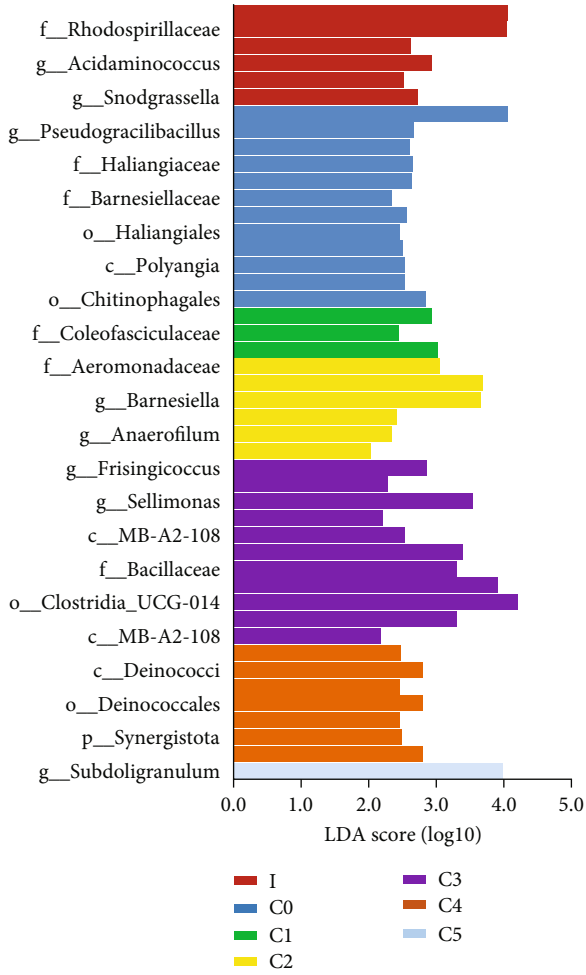

Figure 9: For the OTUs with significant differences in abundance in different groups fed diets with gradient levels of functional oligosaccharides and their effects, the LDA scores (≥2) are listed, and a higher score means larger effects.

of muscle development-related genes [50]. The hardness, gumminess, and chewiness of muscle were higher in the addition groups than in the no addition control group, which indicated that proper addition of functional oligosaccharides may promote muscle texture indices.

The effect of functional oligosaccharide addition was more evident when the culture condition was poor and the growth performance was greatly affected by intestinal health [51, 52]. The experimental conditions in the present study were relatively good and without external stress, and the growth also increased; moreover, the FCR significantly decreased. These results might be mainly attributed to the increase in nutrient absorption. Functional oligosaccharides could provide a better internal environment for the intestinal tract and enhance intestinal health by improving intestinal tissue and increasing digestive enzyme activity and the production of short-chain fatty acids [53].

The common carbohydrates found in fish feeds are glucose, fructose, starch, and sucrose. High dietary carbohydrates always lead to excessive hepatic glycogen accumulation and impaired hepatocyte function, further producing negative effects on the growth and health status of fish, especially carnivorous species such as *M. salmoides* [54–56]. The growth rate and feed efficiency were improved after the addition of functional additives with a relatively low content of fish meal in diets [57, 58]. With the addition of oligosaccharides to a high carbohydrate diet, the flesh quality was improved due to the elevated water holding capacity, texture, and nutritional value of amino acids [59]. Additionally, the addition of functional oligosaccharides made muscles tighter and more elastic in the present study.

In the present study, there were significant differences in growth and FCR when *M. salmoides* were fed a gradient of functional oligosaccharide supplementation. The group fed 1.0 mg/kg functional oligosaccharides showed the highest growth rate and lowest FCR. The effective dosage was lower than that required for most fish according to previous studies. This might be due to the combination of different functional oligosaccharides further enhancing the application effects compared to individual addition [60, 61]. However, the proportion of various functional oligosaccharides should be optimized for more effective utilization [62]. Moreover, dosage and safety analyses should be further performed for various species, growth periods, and culture environments [45, 63].

Nondigestible carbohydrates could also alleviate metabolic syndrome, reduce inflammation, enhance the immune response, and improve the intestinal mucosal barrier by regulating the intestinal microbiota. The protective effects are attributed to the maintenance of mucosal barrier function and inhibition of immune injury via regulation of the gut microbiota [64, 65]. Moderate fiber supplementation regulated the intestinal microbiota in largemouth bass, reduced the abundance of Fusobacteria, and increased the abundances of Firmicutes and Proteobacteria [66]. Functional oligosaccharides were deemed to contribute to the proliferation of beneficial bacteria such as *Bifidobacterium*, *Lactobacillus*, and *Prevotella* [67] and inhibition of the growth of potentially harmful bacteria such as *Aeromonas*, *Ruminococcus*, and *Enterobacter* [68, 69]. In the present study, the abundance of the phyla Firmicutes and Actinobacteria increased, while that of the phylum Bacteroidetes decreased with oligosaccharide addition. Both the phyla of Firmicutes and Bacteroidetes were more abundant in the 1.0 mg/kg addition group than in the other groups, which suggested a higher decomposition ability of cellulose, protein, and polysaccharides. At the genus level, the genera *Bacteroides* and *UCG-014* were considerably higher in the 1.0 mg/kg addition group, which might play a role in stabilizing the intestinal enteric environment and reducing inflammation. However, the relative abundance of *Bifidobacterium* was higher in the control group than in the experimental groups, and there was no significant difference among groups with different addition levels. In contrast, the relative abundance of *Pseudomonas* was higher with high oligosaccharide addition (2.0 mg/kg), which indicated a high risk of inflammation with excess addition of functional oligosaccharides. The functional prediction for gut microbes also indicated a significant positive correlation between the oligosaccharide addition level and nutrient metabolism, especially carbohydrate and energy metabolism. Overall, the addition of functional oligosaccharides to the diet of *M. salmoides* had effects on feeding, physiology, intestinal morphology, and microbial communities.

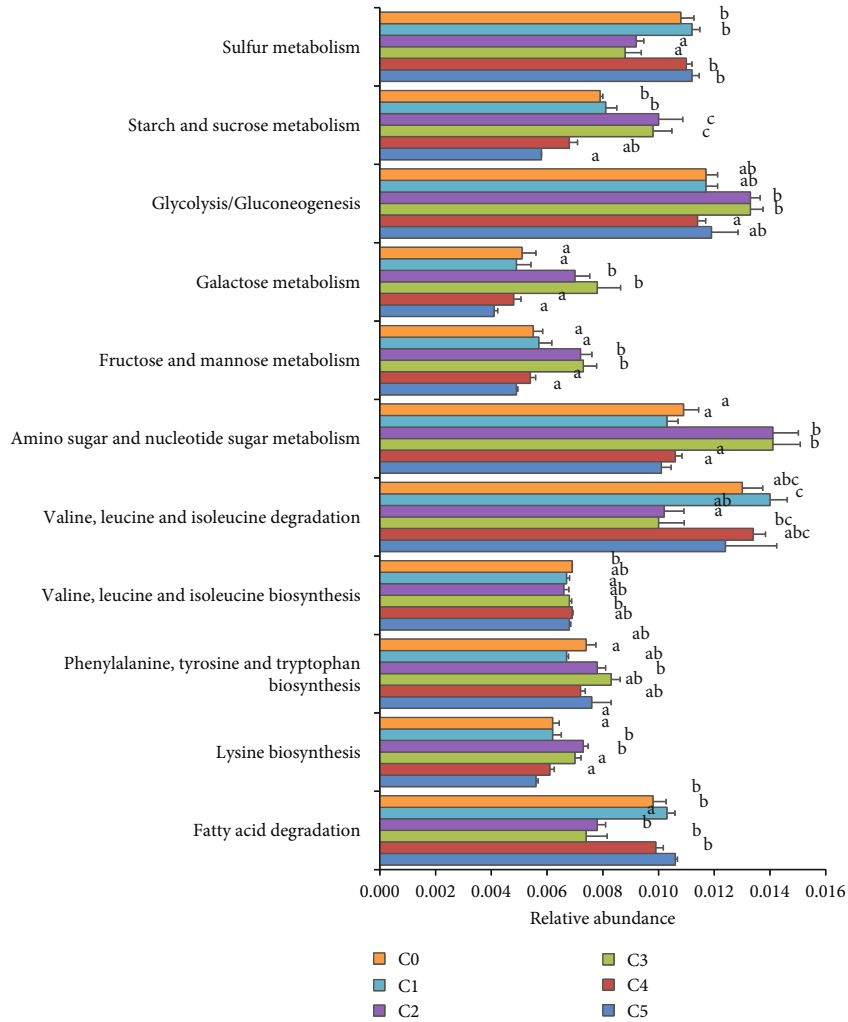

Figure 10: Predicted functional categories and pathways in KEGG level 3 for groups fed diets with gradient levels of functional oligosaccharides. The different letters next to the bars indicate significant differences ($P < 0.05$).

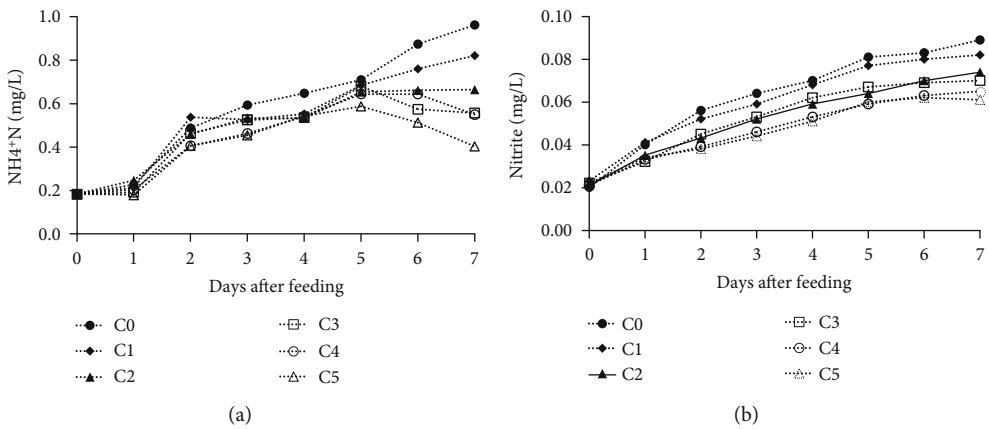

Figure 11: Variation in ammonia-nitrogen (a) (NH_4^+N) and nitrite (b) during 7 d after feeding with diets with a gradient level of functional oligosaccharides.

5. Conclusion

Growth, feed efficiency, biochemistry, physiology, visceral histology, and gut microbiota were all affected by supplementation with functional oligosaccharides. The proper addition of functional oligosaccharides promoted growth, decreased the feed conversion ratio, and increased the hardness, springiness, resilience, cohesiveness, gumminess, and chewiness of muscle. Additionally, functional oligosaccharides promoted microbial abundance related to carbohydrate metabolism. A suitable combination of different functional oligosaccharides could reduce the effective dosage needed, while excessive addition may cause damage to the morphology and function of the liver and gut.

Data Availability

The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (accession number: PRJNA728850) (http://trace.ncbi.nlm.nih.gov/Traces/sra/).

Conflicts of Interest

There are no conflicts of interest to declare.

Acknowledgments

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