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Research Article

Effects of Dietary Sodium Propionate on Growth Performance, Fillet Texture, Hematologic and Plasma Biochemical Parameter, Immune Responses, and Intestine Histology of Juvenile *Trachinotus ovatus*

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Seven graded levels of sodium propionate (SP) diets with 0 (SP1), 0.2% (SP2), 0.4% (SP3), 0.6% (SP4), 0.8% (SP5), 1.0% (SP6), and 1.2% (SP7) were prepared to feed *Trachinotus ovatus* (initial body weight: 8.64 ± 0.08 g) for 56 days. The results showed that increasing dietary SP levels quadratically increased significantly final body weight (FBW), weight gain rate (WGR), and specific growth rate (SGR) of *T. ovatus* but linearly and quadratically decreased significantly viscerosomatic index (VSI) and hepatosomatic index (HSI) of *T. ovatus* ($P<0.05$). In the SP4 treatment, FBW, WGR, and SGR presented the highest values. Both positive linear and quadratic trends were detected between crude lipid content of whole fish, adhesiveness of dorsal muscle, white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), blood performance, high-density lipoprotein cholesterol (HDL-c), intestinal villus height, and dietary SP level, while negative linear and quadratic trends were found between firmness of dorsal muscle, triglyceride (TG), glucose (GLU), and dietary SP level ($P<0.05$). The increasing SP led to quadratic increases in lymphocyte (Lym), complement 3 (C3), chymotrypsin, villus number, and muscle layer thickness, and a quadratic decrease in hepatic malondialdehyde (MDA$P<0.05$). A significant negative linear trend was found between the content of glutamic-pyruvic transaminase (GPT) and dietary SP level, while significant positive linear trends were presented between C4, immunoglobulin M (IgM), α-amylase and dietary SP level ($P<0.05$). The increasing SP resulted in linear and quadratic increases in superoxide dismutase (SOD), total antioxidant capacity (T-AOC) of livers and C3, C4, IgM of head kidney ($P<0.05$). The expression levels of tumor necrosis factor alpha (*TNF-α*) and interleukin-8 (*IL-8*) were linearly and quadratically decreased, while the mRNA levels of growth factor beta (*TGF-β*) were linearly and quadratically increased with the increasing SP level ($P<0.05$). In conclusion, SP could be considered as a beneficial feed additive for enhancing growth and immunity of fish. And dietary SP level at 0.6% is optimal for the growth of *Trachinotus ovatus* based on a quadratic regression model of WGR.

1. Introduction

Trachinotus ovatus is a carnivorous and marine fish, which has become popular because of its delicious taste and high-nutritional value in recent years [1]. In 2022, *T. ovatus* became the second largest marine-farmed fish in China, with a production of approximately 245-thousand tons, second only to that of Large yellow croaker (*Larimichthys crocea*) [2]. However, intensive culture pattern often caused the inflammation and disease of *T. ovatus*, which led to huge

damage for farmers. In order to cut disease-related pecuniary loss, the study of boosting immune response and disease resistance by using specific nutrients or other dietary compounds has attracted growing interests [3]. As "safe dietary compounds," short-chain fatty acids (SCFAs) have been taken into account in aquatic study.

SCFAs are the fatty acids with 1–6 carbons, primarily containing acetate, propionate, and butyrate [4]. SCFAs act a vital role in the intestinal health of host [5]. First, SCFAs are absorbed by intestinal epithelia and provide energy for the development of intestinal epithelial cells [6]. In addition, SCFAs can restrain the amount of pathogen via enhancing the acidity of the enteric cavity [7, 8]. Anti-inflammatory, antioxidative, and anti-cancer are also important properties of SCFAs [9]. Propionate and its salts are one of the main SCFAs. Several evidences have demonstrated that sodium propionate (SP) can bring extensively beneficial effects for the health of aquaculture fish such as beluga sturgeon (*Huso huso*) [10], Caspian white fish (*Rutilus frisii kutum*) [11], common carp (*Cyprinus carpio* L.) [12], zebra fish (*Danio rerio*) [13, 14], European seabass (*Dicentrarchus labrax*) [15], and goldfish (*Carassius auratus*) [16].

In view of the benefits of SP in various fish, it is worth expecting that SP could improve the growth and immunity of *T. ovatus*. Consequently, this study was conducted to evaluate the effects of SP on *T. ovatus* based on growth indicators, fillet quality, hematologic and plasma biochemical parameters, intestine morphology, and immune response.

2. Materials and Methods

2.1. Ethics Statement. All protocols were approved by the Laboratory Animal Care Committee of Xinyang Agriculture and Forestry University (Xinyang, China).

2.2. Experimental Feed. A basal diet was designed including 44% protein and 10% lipid. The formulation of the basal diet was presented in Table 1. Seven isoproteic and isolipidic diets were prepared by inclusion of graded levels (0%, 0.2%, 0.4%, 0.6%, 0.8%, 1.0%, and 1.2%) of SP (purity ≥ 99.0%, Shanghai Sangon Biotech Co., Ltd.), which were named as SP1, SP2, SP3, SP4, SP5, SP6, and SP7, respectively. The addition levels of SP were selected according to these studies [12, 16]. To make the chemical components as identical as possible, the increment of SP was the substitute for the decrement of zeolite powder. The manufacture and storage of feed according to our previous study [17].

2.3. Fish Husbandry. The experimental fish were acquired from Long Qizhuang Farming (Shenzhen, China). The feeding trial was performed in the polythene cages. Juvenile *T. ovatus* was fed basal diets for 14 days to acclimatize the experimental environmental. After the acclimation, a batch of 525 uniform-sized fish (8.64 ± 0.08 g) were allocated into 21 polythene cages (1.8 m^3; 25 fish per cage) in a pond. Each diet was randomly assigned to triplicate cages. During the whole experiment, *T. ovatus* was hand-fed twice a day (7:00 and 18:00) until apparent satiation. The information of mortality and feed intake every day was collected for analyzing mortality rate and feed efficiency ratio (FER). The results of water environment condition were presented as follows: temperature 30.98 ± 0.38°C, salinity 17.25 ± 0.45‰, pH 7.75 ± 0.11, dissolved oxygen 7.42 ± 0.18 mg/L, ammonia ≤ 0.05 mg/L, and nitrite ≤ 0.01 mg/L.

Table 1: Formulation and proximate analysis of the basal diets (air dry weight).

Ingredients[a]	Content (%)
Fish meal	25.0
Soybean meal	15.0
Soy protein concentrate	10.0
Peanut meal	10.0
Pork powder	6.0
Brewers dried yeast	5.0
Wheat flour	18.0
Fish oil	6.0
Soybean Lecithin	1.0
Vitamin and mineral mix[b]	1.0
Choline chloride	0.5
Monocalcium phosphate	0.5
L-methionine	0.2
Antioxidant	0.1
Attractant	0.5
Zeolite powder	1.2
Proximate composition	
Moisture	9.34
Crude protein	44.61
Crude lipid	10.98
Ash	9.82

[a]Fish meal, soybean meal, peanut meal, wheat flour, soybean lecithin, vitamin and mineral mix, choline chloride, monocalcium phosphate, attractant, zeolite powder were purchased by Kingkey Smart Agri Technology Co., Ltd. (Shenzhen, China). Soy protein concentrate, brewers dried yeast, antioxidant were purchased by Guangdong Yuequn Ocean Biological Research Development Co., Ltd. (Jieyang, China). Pork powder weas purchased by Guangdong Xunyuan Nutrition Technology Co., Ltd. (Jieyang, China). Fish oil was purchased by Yongxing Concentrated Feed Co., Ltd. (Guangzhou, China). L-methionine was purchased by Changyi Pharmaceutical Co., Ltd. (Zhejiang, China). [b]Vitamin premix provided the following per kg of diet: VB_2 45 mg, pantothenic acid 60 mg, VB_{12} 0.1 mg, VK_3 10 mg, inositol 800 mg, nicotinic acid 200 mg, folic acid 1.2 mg, biotin 32 mg, VD_3 5 mg, VE 120 mg, VC 2.0 g, choline chloride 2.0 g, ethoxyquin 150 mg, avicel 14.52 mg. Mineral premix provided the following per kg of diet: NaF 4 mg, KI 1.6 mg, $CoCl_2 \cdot 6H_2O$ (1%) 100 mg, $CuSO_4 \cdot 5H_2O$ 20 mg, $FeSO_4 \cdot H_2O$ 160 mg, $ZnSO_4 \cdot H_2O$ 100 mg, $MnSO_4 \cdot H_2O$ 120 mg, $MgSO_4 \cdot 7H_2O$ 2.4 g, $Ca(H_2PO_4)2 \cdot H_2O$ 6.0 g, NaCl 200 mg, and zeolite powder 30.90 g.

2.4. Sampling. The eugenol (100 mg/L) was used to bring fish to an anesthetic state after fish being fasted for 24 hr. The total amount and weight of experimental fish from each cage were enregistered for calculating survival rate and weight gain rate (WGR). A total of eight fish from each cage were randomly sacrificed. Three of them were frozen at −20°C for the determining of whole-body composition. The weight and length of the remaining *T. ovatus* were measured for calculating the condition factor (CF). Then, *T. ovatus* were drawn blood via the tail vein using 1.0 mL heparin-treated syringes. The small amount of blood (0.5 mL) was quickly transferred

to anticoagulated tubes for measuring hematological parameters. The rest of blood samples was used to obtain plasma through the centrifugation at 3,000 r for 15 min at 4°C and maintained at −80°C until needed. Next, the viscera and livers of these fish were weighed for analyzing the viscerosomatic index (VSI) and hepatosomatic index (HSI). Finally, liver, head kidney, and intestine were extracted and frozen at −80°C till use.

Another three fish from each cage were captured. The dorsal muscle was removed for analyzing the fillet quality. The midgut was obtained and soaked in 4% paraformaldehyde for histology observation.

2.5. Growth Performance.

$$\mathrm{WGR}\,(\%) = 100\% \times \frac{W_b - W_a}{W_a}, \quad (1)$$

$$\mathrm{SGR}\,(\%/\mathrm{day}) = 100\% \times \frac{\mathrm{Ln}W_b - \mathrm{Ln}W_a}{N_a}, \quad (2)$$

$$\mathrm{FER} = \frac{W_d}{W_c}, \quad (3)$$

$$\mathrm{CF}(\mathrm{g/cm^3}) = 100\% \times \frac{W_e}{L_a^3}, \quad (4)$$

$$\mathrm{VSI}(\%) = \frac{W_f}{W_e}, \quad (5)$$

$$\mathrm{HSI}(\%) = \frac{W_g}{W_e}, \quad (6)$$

$$\text{Survival rate}\,(\%) = 100\% \times \frac{N_b}{N_c}. \quad (7)$$

W_a, W_b, W_c, W_d, W_e, W_f, W_g, L_a, N_a, N_b, and N_c are, respectively, indicated as initial body weight (g), final body weight (g), dry diet feed (g), wet weight gain (g), body weight (g), viscera weight (g), liver weight (g), body length (cm), number of days, and finial and initial number of fish.

2.6. Proximate Composition. Body composition was tested based on our previous method [17]. In a word, moisture was measured at 105°C for 48 hr; crude protein was analyzed using an Auto Kjeldahl™ 2300 System; crude lipid was determined using Soxtec™ 2055 (FOSS); and ash was assessed by a muffle furnace (FO610C) at 550°C for 10 hr.

2.7. Texture Profile Analysis (TPA). TPA of dorsal muscle samples was determined using the Universal Texture Analyzer (TC3, Brookfield, USA). The textural parameters such as gumminess, chewiness, and firmness were tested with double compression. The parameters of analyzer were set as follows: TA44 spherical probe, the displacement 3 mm, test speed 0.1 cm/s, and trigger values 5G unit.

2.8. Hematological and Biochemical Indices. White blood cell (WBC), red blood cell (RBC), lymphocyte (Lym), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) of blood were analyzed by auto hematology analyzer (Mindray, BC-5000 Vet). Total cholesterol (TC), total protein (TP), triglyceride (TG), glucose (GLU), high-density lipoprotein cholesterol (HDL-c), glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), and alkaline phosphatase (ALP) of plasma were determined through ROCHE-P800 automatic biochemical analyzer. The blood performance was calculated according to the method [18].

$$\text{Blood performance} = \mathrm{lnWBC} + \mathrm{lnRBC} + \mathrm{lnHGB} + \mathrm{lnHCT} + \mathrm{lnTP}. \quad (8)$$

2.9. Enzyme Activities Assay. Liver, head kidney, and intestine were homogenized in the precooled homogenate medium (1 : 9, *w/v*). The supernatant was obtained after the centrifugation of homogenates (3,000 r, 4°C) for 20 min. The superoxide dismutase (SOD), total antioxidant capacity (T-AOC), malondialdehyde (MDA) levels of the liver, and the α-amylase, chymotrypsin, lipase activities of the intestine, and the C3, C4, immunoglobulin M (IgM) contents of the plasma and head kidney were calculated using the corresponding kits (Nanjing, China) through the Absorbance Microplate Reader (VersaMax™, SMP500-18170-OUJL, USA).

2.10. Intestinal Morphology. Immobilized segments of the intestine were soaked at graded ethanol levels for the dehydration. Embedded process of paraffin wax and H&E staining were conducted in Google Biotechnology Ltd., Morphological indicators were examined with the CaseViewer.

2.11. Quantitative Real-Time PCR. The mRNA level of immune-related genes was determined on Roche LightCycler® II 480 system (Roche Diagnostics, Shanghai, China). The process of RNA extraction, cDNA synthesis and qPCR analysis has been described by our previous approaches [17]. β-Actin was used as the housekeeping gene. The primers were synthetized by Ruibo Biotechnology Co., Ltd. (Table 2). The qPCR analysis was performed with a 12.5 μL reaction solution through Roche LightCycler® II 480 system (Roche Diagnostics). The reaction program was set as 95°C for 30 s, 40 cycles at 95°C for 5 s, and an annealing step at 60°C for 30 s. The mRNA levels were assessed by the $2^{-\Delta\Delta Ct}$ method [21]. Three biological repetitions were conducted for each treatment.

2.12. Data Analysis. The outcomes were displayed as means ± SEM. The normality and homoscedasticity of the data were verified before analysis. Significance was subjected to one-way ANOVA followed by Tukey's tests using SPSS 23.0 software. Orthogonal polynomial contrasts were performed to determine whether the trend of the effect was linear and/or quadratic [22]. Significant difference was set at $P < 0.05$.

3. Results

3.1. Growth Performance. With increasing dietary SP level, FBW, WGR, and SGR of *T. ovatus* significantly increased

Table 2: Targeted gene primer sequences used for qPCR analysis.

Genes		Sequence	Source
TGF-β	F	TATCCCTCTACAACAGCACCA	[19]
	R	GGTCAGCAGGCGGTAATC	
IL-8	F	GAGAAGCCTGGGAATGGA	[19]
	R	GAGCCTCAGGGTCTAAGCA	
TNF-α	F	CGCAATCGTAAAGAGTCCCA	[19]
	R	AAGTCACAGTCGGCGAAATG	
IL-10	F	CGTCCTGGCTCTCTTGTCTCTCCTC	[19]
	R	TGTCCATGTCATTGTTTGCCTCATA	
ZO-1	F	CGACAAAGAGAAAGGAGAAACG	[20]
	R	AGAAGTGGTCAATGAGCACAGATA	
Claudins	F	CCCTGCTGGTGTTTGACTAT	[20]
	R	GCAGTCGTAATCCTGGTCTC	
β-Actin	F	TGAACCCCAAAGCCAACAGG	[19]
	R	CCGCAGGACTCCATACCAAG	

TNF-α, Tumor necrosis factor alpha; *IL-8*, Interleukin-8; *TGF-β*, Transforming growth factor beta; *IL-10*, Interleukin-10; *ZO-1*, Zonula occludens protein 1.

quadratically (Table 3, $P<0.05$). FBW, WGR, and SGR increased in SP2, SP3, and SP4 treatments but then decreased in SP5, SP6, and SP7 treatments. The highest FBW, WGR, and SGR was recorded in the SP4 treatment. Both VSI and HSI of *T. ovatus* significantly decreased linearly and quadratically with increasing dietary SP level ($P<0.05$). Nonsignificant difference was seen in FER, CF, and survival rate after *T. ovatus* was fed seven diets for 8 weeks ($P>0.05$). The optimal supplement level of SP for the best growth of *T. ovatus* was detected to be 0.6% according to a quadratic regression model of WGR (Figure 1).

3.2. Whole-Body Composition and Fillet Quality. Dietary SP levels significantly influenced the lipid contents of the whole fish as well as the firmness and adhesiveness of the dorsal muscle (Table 4, $P<0.05$). With increasing dietary SP levels, the lipid contents of the whole fish significantly increased linearly and quadratically, while the firmness of dorsal muscle significantly decreased linearly and quadratically ($P<0.05$). A significant positive linear trend was found between the adhesiveness of dorsal muscle and dietary SP level ($P<0.05$). There were no significant discrepancies in crude protein and ash of the whole fish as well as elasticity, chewiness, and gumminess of dorsal muscle among all groups ($P>0.05$).

3.3. Hematological Parameters. The results of hematology are presented in Table 5. The concentrations of WBC, RBC, and HGB showed positive linear and quadratic relationship with dietary SP level ($P<0.05$). WBC content of SP5 and SP6 treatments was significantly higher than that of other treatments ($P<0.05$). Barring SP2, the treatments supplemented SP had a significantly higher RBC level than SP1 ($P<0.05$). HGB significantly augmented in the SP2, SP4, SP5, and SP6 treatments ($P<0.05$). Lym contents significantly increased linearly ($P<0.05$). The highest of HCT was emerged in the SP4 treatment ($P<0.05$). With increasing dietary SP levels, blood performance significantly increased linearly and quadratically ($P<0.05$). The blood performance of SP4, SP5, and SP6 treatments was significantly higher than that of the SP1 ($P<0.05$). Supplementation of dietary SP did not significantly influence the MCV, MCH, and MCHC of blood ($P>0.05$).

3.4. Plasma Biochemistry and Immune Parameters. As shown in Table 6, TG, GLU, HDL-c, GPT, C3, C4, and IgM of plasma were significantly influenced by dietary SP levels ($P<0.05$). With increasing dietary SP levels, TG and GLU of plasma significantly decreased linearly and quadratically, while HDL-c of plasma significantly increased linearly and quadratically ($P<0.05$). TG of the SP3, SP5, SP6, and SP7 treatments presented significantly lower than that of the SP1 treatment. GLU of plasma in the SP supplementation treatments was lower, while the HDL-c in the SP supplementation treatments was higher than that of the SP1 treatment ($P<0.05$). A significant negative linear relationship was found between GPT and dietary SP level ($P<0.05$), and a significant positive linear trend was presented between C4, IgM, and dietary SP level ($P<0.05$). With increasing dietary SP levels, C3 of plasma significantly increased quadratically ($P<0.05$). C3 content in SP4 was significantly higher than that in SP1 ($P<0.05$). SP addition did not significantly influence the TC, TP, and GOT of plasma ($P>0.05$).

3.5. Antioxidative and Immune Parameters. The results are displayed in Table 7. Both linear and quadratic effects were observed in SOD and T-AOC of liver as well as C3, C4, and IgM of head kidney ($P<0.05$). Hepatic SOD in SP7 and T-AOC in SP3, SP4, SP5, SP6, and SP7 were significantly higher than that in SP1 ($P<0.05$). C3, C4, and IgM of head kidney increased in SP2, SP3, and SP4 treatments and then decreased in SP5, SP6, and SP7 treatments. The highest values of the three parameters were observed in the SP4 treatment. Hepatic MDA significantly decreased quadratically ($P<0.05$) and the lowest was recorded in the SP4 treatment.

3.6. Digestive Enzymes and Morphology in Intestine. The results are presented in Figure 2. Dietary SP4 significantly elevated lipase activity compared to SP1 ($P<0.05$). The α-amylase activity significantly increased linearly, while the chymotrypsin activities significantly increased quadratically ($P<0.05$). Chymotrypsin activities in SP4 and SP6 were markedly higher than those in SP1 ($P<0.05$). The results of H&E staining showed moderate SP level in diets could improve intestinal histomorphology in *T. ovatus* (Figure 3). With increasing dietary SP levels, a significant positive quadratic effect was found between villus number, muscle layer thickness (MLT) and dietary SP level ($P<0.05$), and the highest values of these parameters were observed in the SP4 treatment. With increasing dietary SP level, intestinal villus height significantly increased linearly and quadratically ($P<0.05$). Villus height in SP2 and SP4 was significantly higher than that in SP1 ($P<0.05$).

3.7. Immunity-Related Gene mRNA Expression. The results are displayed in Figure 4. Both linear and quadratic effects were found in the mRNA level of *TNF-α*, *IL-8*, and *TGF-β* genes ($P<0.05$). The expression levels of *TNF-α* and *IL-8* genes in SP3, SP4, SP5, SP6, and SP7 were significantly lower than those in SP1 ($P<0.05$). The mRNA level of *IL-10*

Table 3: Effects of dietary SP levels on growth performance in *T. ovatus*.

Parameters	Dietary SP supplement levels							Pr > F		
	SP1 (0)	SP2 (0.2%)	SP3 (0.4%)	SP4 (0.6%)	SP5 (0.8%)	SP6 (1.0%)	SP7 (1.2%)	ANOVA	Linear trend	Quadratic trend
IBW (g)	8.67 ± 0.05	8.64 ± 0.04	8.68 ± 0.02	8.58 ± 0.03	8.58 ± 0.07	8.67 ± 0.03	8.68 ± 0.07	0.537	0.992	0.157
FBW (g)	60.59 ± 1.08^{a}	64.02 ± 0.45^{ab}	66.24 ± 0.32^{bc}	70.64 ± 0.74^{d}	68.42 ± 0.82^{cd}	64.40 ± 0.90^{abc}	60.61 ± 1.19^{a}	0.000	0.512	0.000
WGR (%)	598.64 ± 8.88^{a}	641.27 ± 1.56^{a}	663.55 ± 5.51^{bc}	723.04 ± 5.95^{d}	697.40 ± 6.55^{cd}	642.37 ± 8.10^{b}	598.42 ± 11.16^{a}	0.000	0.379	0.000
SGR (%/d)	3.47 ± 0.02^{a}	3.58 ± 0.00^{a}	3.63 ± 0.01^{bc}	3.76 ± 0.01^{d}	3.71 ± 0.01^{cd}	3.58 ± 0.02^{b}	3.47 ± 0.03^{a}	0.000	0.413	0.000
FER	0.68 ± 0.01	0.69 ± 0.01	0.65 ± 0.02	0.68 ± 0.00	0.68 ± 0.01	0.69 ± 0.00	0.69 ± 0.01	0.358	0.331	0.256
VSI (%)	6.64 ± 0.12^{c}	5.92 ± 0.15^{ab}	5.78 ± 0.02^{ab}	6.18 ± 0.11^{bc}	5.66 ± 0.18^{ab}	5.44 ± 0.05^{a}	6.14 ± 0.15^{bc}	0.000	0.001	0.000
HSI (%)	1.25 ± 0.03^{d}	0.93 ± 0.01^{b}	0.91 ± 0.01^{b}	1.08 ± 0.02^{c}	0.83 ± 0.02^{ab}	0.76 ± 0.02^{a}	1.05 ± 0.01^{c}	0.000	0.000	0.000
CF (g/cm^3)	3.37 ± 0.03	3.29 ± 0.04	3.28 ± 0.01	3.44 ± 0.09	3.41 ± 0.01	3.32 ± 0.02	3.29 ± 0.08	0.240	0.903	0.314
Survival (%)	100 ± 0.00	100 ± 0.00	98.67 ± 1.33	98.67 ± 1.33	98.67 ± 1.33	100 ± 0.00	98.67 ± 1.33	0.798	0.466	0.482

IBW, initial body weight; FBW, final body weight; WGR, weight gain rate; SGR, specific growth rate; FER, feed efficiency ratio; CF, condition factor; VSI, viscerosomatic index; HSI, hepatosomatic index. Mean values within the row unlike superscript letters were significantly different ($P < 0.05$). Significance probability associated with the F-statistic.

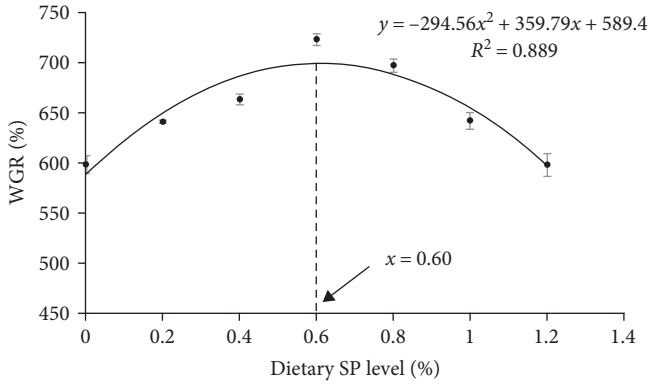

FIGURE 1: Relationship between dietary sodium propionate (SP) and weight gain rate (WGR). Each point is means ± SEM of three replicate groups, with 25 fish per group.

significantly increased with a positive quadratic trend, and the mRNA level of *IL-10* in SP5 was significantly higher than that in SP1 ($P<0.05$). Among the groups, the mRNA level of *claudins* gene in SP4 was the highest. No significant difference was observed in *ZO-1* ($P>0.05$).

4. Discussion

The research about the potential influences of SCFAs on aqueous animals has become the hot spot of fish nutritionists [23–25]. SP, as one of the main SCFAs, has been confirmed to be potent benefits on the growth and digestion for aquaculture animals. In the current study, moderate SP level in diets observably promoted the WGR and SGR of *T. ovatus*. Similar phenomena were also found by the studies on beluga sturgeon [10], Caspian white fish [11], and goldfish [16]. However, growth of *T. ovatus* in SP7 group showed an obviously decreased trend. A recent study demonstrated that addition of excess SCFAs changed the acidity of the feed and caused a decreased palatability [26], which supported our result. Intestine is a chief organ for digestion and assimilation in fish. Previous studies have confirmed SCFAs (potassium diformate, sodium acetate, and sodium butyrate) had ameliorative impacts on digestive capacity of fish [25–27]. In our study, enhanced activities of chymotrypsin, α-amylase and lipase, meliorative intestine morphology, and increased villus number, villus height, and MLT were found in *T. ovatus* fed SP4 diets. Digestive enzymes and macro-morphometric parameters of intestine could directly reflect physiologic and metabolic status of the body [28, 29]. Therefore, we conjectured that dietary SP heightened the metabolism of nutrients by enhancing the activities of digestive enzymes and macro-morphometric parameters, thereby promoting growth performance of *T. ovatus*. VSI and HSI are regarded as the vital index reflecting viscus and liver status. In general, high VSI and HSI implied the intensification in visceral lipid deposition and the risk of developing fatty liver disease, respectively [30, 31]. Up to now, the effect of SP on VSI and HSI in fish is unclear. Previous studies have revealed potassium diformate could decrease VSI and HSI of *T. ovatus* and Nile tilapia (*Oreochromis niloticus*) [26, 32]. In the current study, dietary SP significantly reduced VSI and HSI of *T. ovatus*, which suggested moderate SP level may defend fish from the danger of liver ailment.

The proximate composition of whole body is a crucial parameter for estimating the quality of aquatic products [33]. Our study showed supplementation of SP significantly increased crude lipid content meanwhile decreased moisture content of whole body. Ahmed et al. [34] demonstrated moisture content is inversely to protein and lipid content, which was consistent with our study. TPA is an important method to assess fillet quality, where firmness, elasticity, chewiness, adhesiveness, and gumminess are the most generally acceptant and researched [35, 36]. Our results displayed adding SP in diets significantly decreased intramuscular firmness and increased intramuscular adhesiveness of *T. ovatus* to a certain degree. Previous studies showed low firmness in the fillet relating to high fat content [37, 38]. Another research also pointed out a direct relationship between succulence, palatability, and the muscle lipid content [39]. These findings suggested that addition of SP in diets improved fillet quality of *T. ovatus*.

Hematology parameters, as pivotal biomarkers evaluating health status of fish were susceptible to nutritional stressors [40, 41]. Leucocytes play a key role in adjusting immunological functions and WBC is generally used to assess the stress levels of aquatic animals [42]. Lym participates in the process of antibody production in teleost fish [43]. The results of present study presented moderate SP level in diets significantly increased the values of WBC and Lym. In addition, our results showed all of the RBC, HGB, and HCT significantly increased in blood of *T. ovatus* fed SP4 diets, which was similar with the studies of goldfish [16] and beluga sturgeon [10]. Previous studies demonstrated that increased RBC, HGB, and HCT were helpful for carrying added oxygen and strengthen the phagocytic activity related to immune [44]. However, these parameters may present inconsistent trends across studies, and it is unscientific to depend on them as health indicators independently. Blood performance as new formula has been confirmed much more reliable and accurate for monitoring fish health and growth [18, 45, 46]. Generally, higher blood performance values indicate better health status [47]. In the present study, the blood performance significantly increased in SP4, SP5, and SP6. Moreover, we calculated and compared the blood performance of fish fed SP diets and control diets from previous studies on beluga sturgeon [10], European seabass [15], and yellowfin seabream (*Acanthopagrus latus*) [48], and found these fish fed SP diets had better growth or/and immune levels while having higher blood performance, which were consistent with our results. These findings suggested moderate SP level was propitious to the growth and health of fish. Besides hematological parameters, plasma biochemistry can be evaluated the nutritional and immune system status of fish. In the present study, the contents of C3, C4, and IgM showed a distinct increase in plasma of fish fed SP4 diets. A study suggested that the increase of the complements and IgM represented the enhancement of body immunity [49]. In this study, decreased TG, GLU, and GPT content and incremental HDL-c content were found in plasma of *T. ovatus* fed SP diets, indicating a hypolipidemic and

Table 4: Effects of dietary SP levels on body composition and texture characteristics of dorsal muscle in *T. ovatus*.

Parameters	Dietary SP supplement levels							Pr > F		
	SP1 (0)	SP2 (0.2%)	SP3 (0.4%)	SP4 (0.6%)	SP5 (0.8%)	SP6 (1.0%)	SP7 (1.2%)	ANOVA	Linear trend	Quadratic trend
Whole-body composition (dry weight)										
Crude protein	54.91 ± 0.27	53.53 ± 0.25	54.03 ± 0.51	54.37 ± 0.72	53.94 ± 0.42	53.93 ± 0.28	53.98 ± 0.10	0.409	0.355	0.431
Crude lipid	31.68 ± 0.20[a]	34.73 ± 0.24[b]	34.03 ± 0.38[b]	33.85 ± 0.40[b]	33.86 ± 0.19[b]	34.17 ± 0.32[b]	34.40 ± 0.24[b]	0.000	0.001	0.006
Ash	12.58 ± 0.16	12.21 ± 0.20	12.19 ± 0.16	11.87 ± 0.23	12.05 ± 0.33	11.91 ± 0.07	11.87 ± 0.29	0.312	0.029	0.335
Moisture	69.76 ± 0.34[b]	66.88 ± 0.17[a]	67.58 ± 0.13[a]	68.70 ± 0.33[ab]	68.05 ± 0.45[ab]	67.26 ± 0.52[a]	68.03 ± 0.80[ab]	0.008	0.114	0.098
Texture characteristics of dorsal muscle										
Firmness (g)	300.83 ± 2.74[c]	295.17 ± 2.24[c]	265.67 ± 4.81[b]	240.83 ± 3.84[a]	243.00 ± 4.93[a]	269.83 ± 2.83[b]	264.17 ± 7.17[b]	0.000	0.000	0.000
Elasticity (mm)	2.16 ± 0.02	2.17 ± 0.02	2.12 ± 0.04	2.14 ± 0.02	2.20 ± 0.07	2.16 ± 0.02	2.19 ± 0.03	0.713	0.449	0.561
Chewiness (mJ)	2.06 ± 0.03	2.08 ± 0.06	2.00 ± 0.04	2.07 ± 0.01	2.00 ± 0.05	2.03 ± 0.04	1.90 ± 0.09	0.249	0.041	0.323
Adhesiveness (mJ)	0.16 ± 0.01[a]	0.17 ± 0.01[ab]	0.16 ± 0.00[a]	0.18 ± 0.01[ab]	0.21 ± 0.00[bc]	0.18 ± 0.02[ab]	0.24 ± 0.01[c]	0.000	0.000	0.090
Gumminess (g)	100.57 ± 1.43	93.7 ± 4.36	96.37 ± 2.51	93.03 ± 4.36	88.6 ± 3.08	91.6 ± 0.56	94.83 ± 1.27	0.188	0.076	0.079

Mean values within the row unlike superscript letters were significantly different ($P < 0.05$). Significance probability associated with the F-statistic.

Table 5: Effects of dietary SP level on hematological parameters in *T. ovatus*.

Parameters	Dietary SP supplement levels							Pr > F		
	SP1 (0)	SP2 (0.2%)	SP3 (0.4%)	SP4 (0.6%)	SP5 (0.8%)	SP6 (1.0%)	SP7 (1.2%)	ANOVA	Linear trend	Quadratic trend
WBC (10^9/L)	0.83 ± 0.06^{a}	0.84 ± 0.01^{a}	0.88 ± 0.03^{a}	0.83 ± 0.03^{a}	1.27 ± 0.01^{b}	1.22 ± 0.01^{b}	0.90 ± 0.03^{a}	0.000	0.000	0.002
Lym (%)	91.60 ± 0.42^{a}	96.97 ± 1.03^{b}	93.70 ± 0.06^{ab}	96.57 ± 0.79^{b}	96.63 ± 0.59^{b}	93.53 ± 1.72^{ab}	95.20 ± 0.78^{ab}	0.007	0.175	0.014
RBC (10^{12}/L)	3.98 ± 0.01^{a}	4.12 ± 0.01^{ab}	4.28 ± 0.02^{b}	4.25 ± 0.01^{b}	4.52 ± 0.07^{c}	4.59 ± 0.05^{c}	4.19 ± 0.04^{b}	0.000	0.000	0.000
HGB (g/L)	159.00 ± 0.00^{a}	166.00 ± 1.00^{bc}	162.00 ± 0.00^{ab}	169.00 ± 0.00^{c}	182.67 ± 1.20^{d}	183.00 ± 2.00^{d}	162.33 ± 0.67^{ab}	0.000	0.000	0.000
HCT (%)	72.50 ± 0.15^{a}	74.07 ± 0.44^{ab}	73.63 ± 0.27^{ab}	78.07 ± 0.19^{b}	75.00 ± 2.37^{ab}	74.00 ± 1.02^{ab}	75.30 ± 0.75^{ab}	0.049	0.102	0.064
MCV (fL)	179.67 ± 1.58	178.03 ± 0.79	181.27 ± 1.39	183.40 ± 0.21	179.13 ± 0.89	177.10 ± 3.80	183.70 ± 0.60	0.104	0.388	0.899
MCH (pg)	39.53 ± 0.35	39.43 ± 0.15	39.90 ± 0.06	39.87 ± 0.03	39.97 ± 0.23	39.70 ± 0.25	39.47 ± 0.19	0.396	0.721	0.051
MCHC (g/L)	219.00 ± 1.00	220.67 ± 1.20	219.00 ± 1.00	217.00 ± 0.57	222.00 ± 5.13	217.00 ± 4.04	221.00 ± 2.00	0.774	0.908	0.718
Blood performance	14.06 ± 0.05^{a}	14.35 ± 0.10^{ab}	14.36 ± 0.08^{ab}	14.55 ± 0.06^{b}	15.02 ± 0.05^{c}	14.97 ± 0.04^{c}	14.36 ± 0.07^{ab}	0.000	0.000	0.000

WBC, white blood cell; RBC, red blood cell; Lym, lymphocyte; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration. Mean values within the row unlike superscript letters were significantly different ($P<0.05$). Significance probability associated with the F-statistic.

Table 6: Effects of dietary SP levels on biochemistry and immune index of plasma in *T. ovatus*.

Parameters	Dietary SP supplement levels							Pr > F		
	SP1 (0)	SP2 (0.2%)	SP3 (0.4%)	SP4 (0.6%)	SP5 (0.8%)	SP6 (1.0%)	SP7 (1.2%)	ANOVA	Linear trend	Quadratic trend
TC (mmol/L)	4.69 ± 0.29	5.26 ± 0.37	5.08 ± 0.55	4.54 ± 0.41	5.27 ± 0.10	5.19 ± 0.34	4.68 ± 0.34	0.635	0.996	0.486
TP (g/L)	33.97 ± 1.17	40.56 ± 3.38	39.13 ± 3.95	44.97 ± 1.58	42.33 ± 1.47	42.13 ± 2.66	37.57 ± 2.42	0.137	0.227	0.013
TG (mmol/L)	3.24 ± 0.80^{a}	1.95 ± 0.02^{ab}	1.53 ± 0.10^{b}	2.05 ± 0.01^{ab}	1.76 ± 0.11^{b}	1.49 ± 0.00^{b}	1.60 ± 0.03^{b}	0.016	0.004	0.046
GLU (mmol/L)	20.52 ± 0.07^{d}	15.69 ± 0.44^{c}	10.83 ± 0.16^{a}	17.19 ± 0.32^{c}	12.64 ± 0.85^{ab}	13.14 ± 0.18^{b}	16.68 ± 0.27^{c}	0.000	0.000	0.000
HDL-c (mmol/L)	2.75 ± 0.03^{a}	3.44 ± 0.11^{bc}	3.76 ± 0.02^{c}	3.22 ± 0.13^{b}	3.44 ± 0.02^{bc}	3.47 ± 0.02^{bc}	3.23 ± 0.11^{b}	0.000	0.015	0.000
ALP (U/L)	63.67 ± 2.03	64.33 ± 2.19	63.67 ± 1.45	66.67 ± 2.73	68.33 ± 0.88	61.33 ± 4.91	56.67 ± 3.18	0.157	0.149	0.030
GPT (U/L)	9.33 ± 1.20^{b}	7.67 ± 1.33^{ab}	4.00 ± 0.00^{a}	6.33 ± 1.20^{ab}	6.67 ± 0.33^{ab}	5.00 ± 1.00^{ab}	5.33 ± 0.88^{ab}	0.028	0.012	0.093
GOT (U/L)	49.33 ± 6.17	52.67 ± 1.45	30.67 ± 4.91	47.00 ± 6.35	43.00 ± 2.31	40.33 ± 5.17	43.00 ± 4.62	0.099	0.233	0.247
C3 (μg/mL)	18.33 ± 1.28^{a}	17.80 ± 1.52^{a}	24.95 ± 1.02^{ab}	32.38 ± 0.89^{b}	18.57 ± 4.50^{a}	18.01 ± 3.39^{a}	19.40 ± 0.64^{a}	0.004	0.825	0.005
C4 (μg/mL)	20.54 ± 2.36^{a}	33.22 ± 0.30^{b}	34.27 ± 2.49^{b}	31.49 ± 3.54^{b}	27.89 ± 0.43^{ab}	29.76 ± 1.21^{ab}	35.12 ± 2.71^{b}	0.005	0.020	0.111
IgM (μg/mL)	22.90 ± 1.33^{a}	34.29 ± 0.69^{ab}	34.64 ± 1.22^{ab}	38.99 ± 1.36^{bcd}	37.94 ± 1.49^{bc}	47.69 ± 0.92^{cd}	50.67 ± 6.05^{d}	0.000	0.000	0.807

TC, total cholesterol; TP, total protein; TG, triglyceride; GLU, glucose; HDL-c, high-density lipoprotein cholesterol; GOT, glutamic-oxalacetic transaminase; GPT, glutamic-pyruvic transaminase; ALP, alkaline phosphatase; C3, complement 3; C4, complement 4; IgM, immunoglobulin M. Mean values within the row unlike superscript letters were significantly different ($P < 0.05$). Significance probability associated with the F-statistic.

Table 7: Effects of dietary SP levels on antioxidative parameters of liver and immune index of head kidney in *T. ovatus*.

Parameters	Dietary SP supplement levels							Pr > F		
	SP1 (0)	SP2 (0.2%)	SP3 (0.4%)	SP4 (0.6%)	SP5 (0.8%)	SP6 (1.0%)	SP7 (1.2%)	ANOVA	Linear trend	Quadratic trend
Liver										
SOD (U/mg prot)	2.26 ± 0.01^{a}	2.26 ± 0.10^{a}	2.30 ± 0.03^{a}	2.26 ± 0.06^{a}	2.27 ± 0.02^{a}	2.49 ± 0.03^{a}	2.97 ± 0.08^{b}	0.000	0.000	0.000
T-AOC (μmol/g prot)	141.09 ± 1.66^{a}	151 ± 5.28^{ab}	199.92 ± 10.66^{cd}	223.67 ± 7.87^{d}	187.99 ± 5.00^{c}	171.23 ± 5.43^{bc}	188.62 ± 0.73^{c}	0.000	0.000	0.000
MDA (nmol/mg prot)	15.13 ± 0.56^{b}	10.86 ± 1.53^{ab}	9.61 ± 0.70^{a}	9.40 ± 0.60^{a}	11.39 ± 0.77^{ab}	13.19 ± 0.90^{ab}	11.31 ± 1.73^{ab}	0.022	0.386	0.006
Head kidney										
C3 (mg/g prot)	15.62 ± 0.33^{a}	23.11 ± 1.98^{ab}	30.04 ± 1.07^{bc}	37.90 ± 2.63^{c}	27.21 ± 1.32^{b}	26.48 ± 1.72^{b}	25.77 ± 1.47^{b}	0.000	0.001	0.000
C4 (mg/g prot)	12.72 ± 0.11^{a}	16.13 ± 1.03^{ab}	20.51 ± 0.52^{cd}	23.76 ± 0.69^{d}	21.52 ± 1.11^{cd}	18.75 ± 0.90^{bc}	18.97 ± 0.63^{bc}	0.000	0.000	0.000
IgM (mg/g prot)	17.48 ± 0.45^{a}	21.67 ± 1.92^{ab}	29.81 ± 0.95^{cd}	46.95 ± 0.84^{e}	36.33 ± 2.27^{d}	33.15 ± 1.14^{d}	25.74 ± 1.86^{bc}	0.000	0.000	0.000

SOD, superoxide dismutase; T-AOC, total antioxidant capacity; MDA, malondialdehyde. Mean values within the row unlike superscript letters were significantly different ($P < 0.05$). Significance probability associated with the F-statistic.

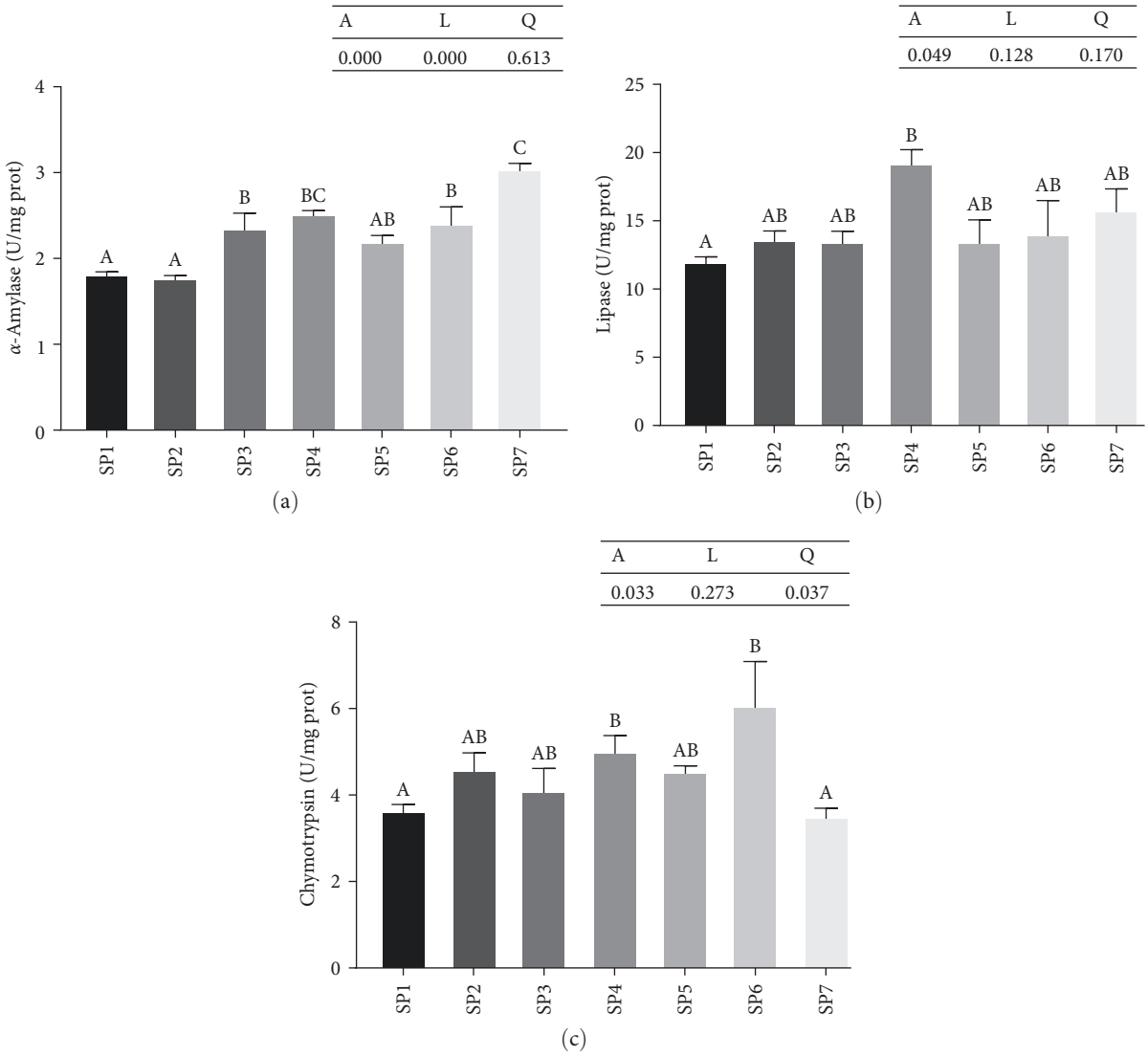

Figure 2: Effects of dietary SP on the digestive enzyme activities (a) α-amylase, (b) lipase, and (c) chymotrypsin in *T. ovatus*. Values represent the mean ± SEM. Different letters above the bar represent significant differences among different groups ($P < 0.05$). A, the variance analyzed by one-way ANOVA; L, linear trend; Q, quadratic trend (similarly hereinafter).

hypoglycemic effect of SP. Hence, we conjectured that SP was beneficial for regulating metabolic and boosting hematogenic immunity.

Besides the role of SP in enhancing blood immunity, our results also found SP significantly increased C3, C4, and IgM contents in head kidney of *T. ovatus*, which suggested SP improved the immune response of head kidney. Furthermore, SP could also activate intestinal immunization to regulate immune response in fish body [16]. Intestinal cytokines (like *TNF-α*, *TGF-β*, *IL-8*, and *IL-10*) participate in activating innate immune defense. Thereinto, *TNF-α* and *IL-8* are pro-inflammatory cytokines, which induced inflammatory response [50]. In contrast, *TGF-β* and *IL-10* as anti-inflammatory cytokines, are connected with the inhibiting of inflammation and the rehabilitating of damage induced by inflammatory response [51]. Previous studies have been reported SCFAs (potassium diformate, sodium acetate, and sodium butyrate) can boost immunity and restrain inflammation process in fish [17, 26, 52]. In the current study, the expression level of *TNF-α* and *IL-8* were downregulated and the expression level of *TGF-β* and *IL-10* presented an increased trend in intestine of *T. ovatus* fed SP diets, indicating enhanced immune responses. Intestinal inflammation was mainly induced by the penetration of pathogenic bacteria from the enteric cavity [53]. Tight junction (TJ) proteins play indispensable roles in the maintenance the intestinal physical barrier [54]. The higher mRNA levels of TJ proteins are involved in the enhancement of physical barrier [8]. Several researches have demonstrated the therapeutical effects on impaired intestinal integrity of SCFAs in fish. For instance, dietary sodium butyrate supplementation upregulated the expression levels of *Occludin*, *ZO-1*, and *claudin-3c* as well as downregulating *claudin-12*, thus retaining the intestine structural integrity of grass carp (*Ctenopharyngodon idella*) [55]. Similarly, dietary sodium acetate and SP upregulated the mRNA level of TJ protein [8]. Our study found addition of SP could obviously

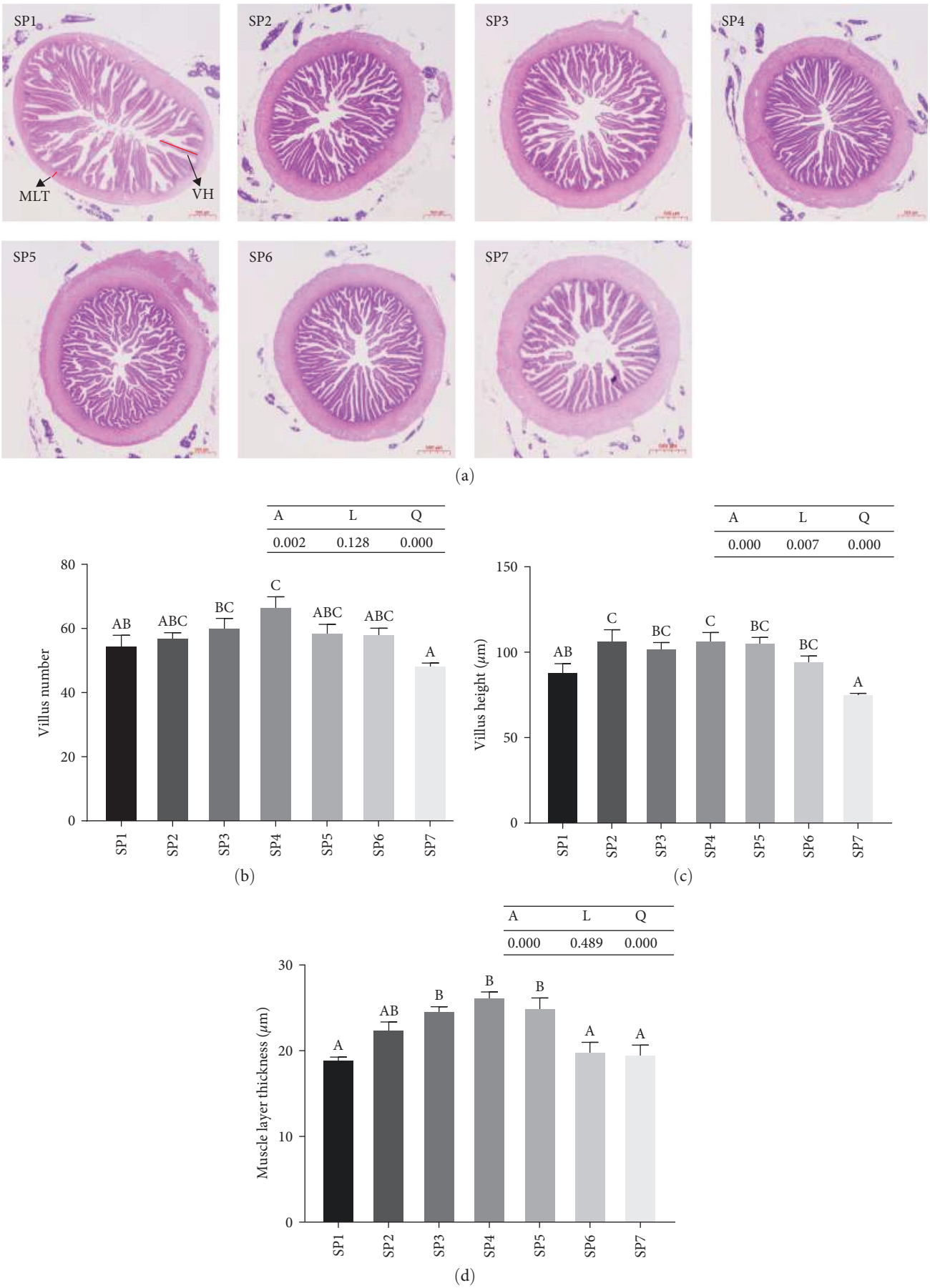

FIGURE 3: Effects of dietary SP level on mid-gut morphology (20x) in *T. ovatus*. (a) The section of H.E. staining. (b–d) Villus number, villus height (VH), and muscle layer thickness (MLT). Different superscript letters indicated significant difference ($P<0.05$).

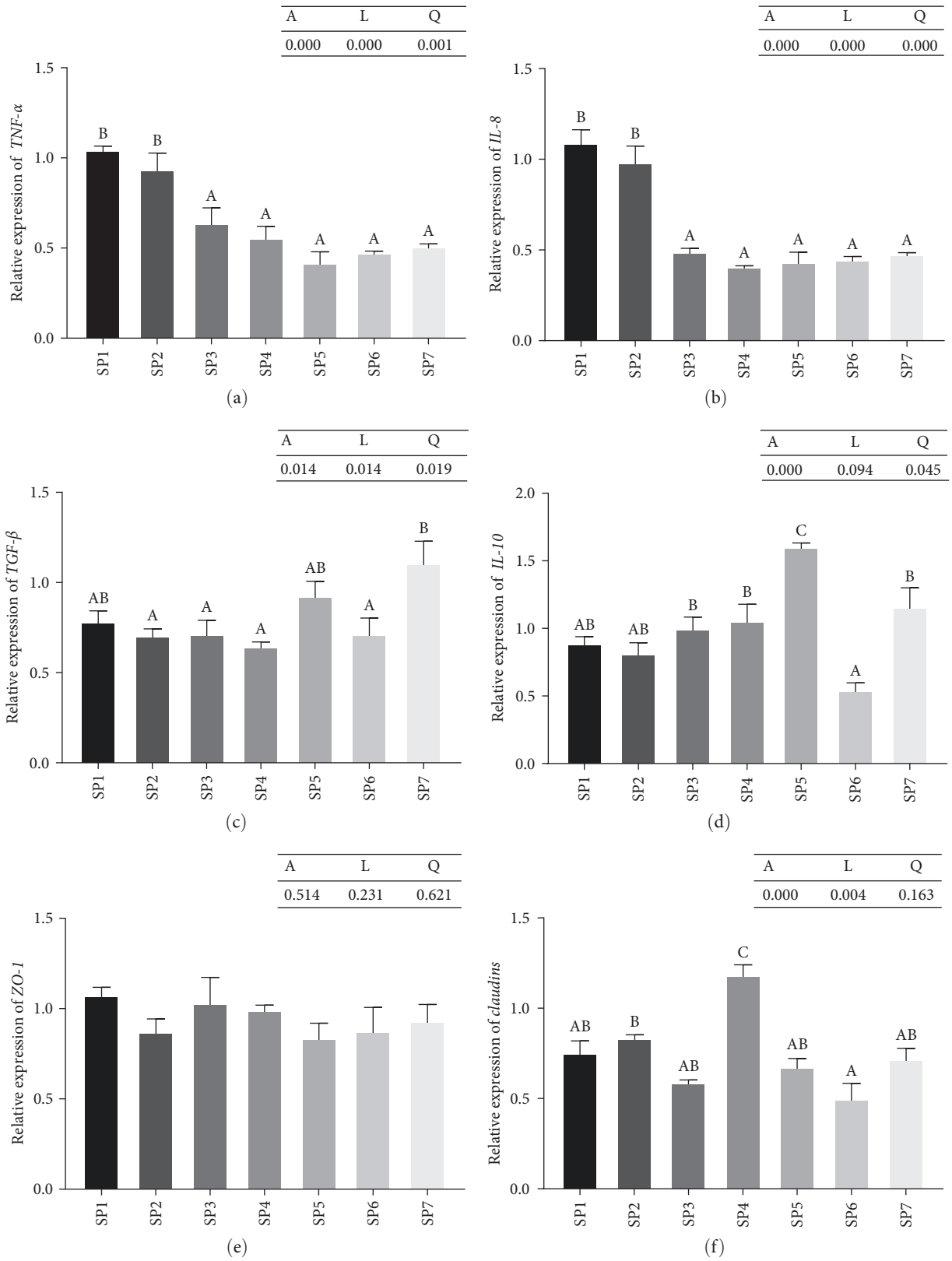

FIGURE 4: Effects of dietary SP on expression of immune-related genes (a) *TNF-α*, (b) *IL-8*, (c) *TGF-β*, (d) *IL-10*, (e) *ZO-1*, and (f) *claudins* in intestine of *T. ovatus*. Values represent the mean ± SEM. Different letters above the bar represent significant differences among different groups ($P < 0.05$).

modulated expression of *claudins* and reached the highest value in SP4 group, which indicated that dietary appropriate SP level could improve the gut barrier function to a certain extent.

Oxidative stress is a main incentive overproducing reactive oxygen species (ROS) [56]. ROS induced lipid peroxidation and produced a lot of MDA, which harmed cellular function [57]. Therefore, the MDA content was a major marker of cell trauma. Antioxidative enzymes act vital roles in the elimination of ROS and T-AOC content represents total antioxidant level of fish [31, 58]. In the present study, distinctly increased T-AOC and decreased MDA contents were found in liver of the SP4 group, suggesting SP had a benefit on relieving oxidative damage. This was supported by the results of beluga sturgeon [10] and common carp [12].

5. Conclusion

Our study demonstrated that moderate supplementation level of SP promoted growth performance through increasing digestive enzyme activities and improving intestinal histology. Dietary supplementation with SP could promote fillet quality by increasing lipid content of fish and improving texture characteristics of dorsal muscle. Additionally, dietary SP can enhance immune and antioxidative capacity of *T. ovatus* through regulating the parameters of hematology and plasma, hepatic antioxidative enzymes, and mRNA expression of intestinal immunology-associated genes. Based on the growth of *T. ovatus*, optimal supplement level of SP is recommended at 0.6%.

Data Availability

Data for this research article were available from the corresponding authors by reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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