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Research Article

Effects of Dietary Arginine on Growth Performance, Digestion, Absorption Ability, Antioxidant Capability, Gene Expression of Intestinal Protein Synthesis, and Inflammation-Related Genes of Triploid Juvenile *Oncorhynchus mykiss* Fed a Low-Fishmeal Diet

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In this study, the effects of arginine on growth, serum antioxidant levels, intestinal digestion physiology, intestinal protein synthesis, and expression of inflammatory-related genes of triploid juvenile rainbow trout (*Oncorhynchus mykiss*) were investigated. Five diets containing 0.86%, 1.65%, 2.10%, 2.85%, and 3.50% arginine were fed to satiation to triplicate groups of 30 fish per tank for 8 weeks. The results showed that the weight gain rate, specific growth rate, and protein efficiency ratio significantly increased. The optimal dietary arginine requirement was 2.48% (5.97% of dietary protein) based on quadratic regression analysis between specific growth rate and dietary arginine content. There were no significant differences in crude lipid and ash content. Moisture significantly decreased, and crude protein content significantly increased ($P < 0.05$). Superoxide dismutase, catalase, and lysozyme levels in serum and the liver first significantly increased and then decreased with the level of arginine compared to the control group ($P < 0.05$), while the malondialdehyde level significantly decreased. Intestinal lipase and amylase levels significantly increased, but there was no significant difference in trypsin level ($P > 0.05$). The muscular layer thickness and villus width foregut in the 2.85% group were significantly increased. TOR at 2.86% and IGF-1 and S6K1 at 2.11% reached the maximum level, and IL-1β, IL-6, and IL-8 showed a decreasing trend with their lowest levels in the 3.50% and 2.85% groups. The expression of TNF-α first decreased and then increased with the arginine level. Both TGF-β and IL-10 expression first decreased and then increased with the level of arginine and reached the maximum value in the 2.85% group. NF-κB showed an opposite trend and reached the lowest value in the 3.50% group. In conclusion, dietary arginine has a significant effect on growth, serum antioxidant capability, digestion physiology, immunity, digestion, and absorption of nutrients in triploid *O. mykiss*, and the optimum requirement is 2.48% fed a low-fishmeal diet.

1. Introduction

In aquaculture, fishmeal provides the majority of dietary protein in feed. However, fishmeal is in short supply and feed manufacturers have begun to replace fishmeal protein with other sources of protein, mainly plant protein concentrates [1]. However, the amino acid composition of plant protein concentrate is different from that of fishmeal protein. Arginine is limited in some plant protein sources, and the requirement for amino acids must be met by an external

supply [2–4]. Arginine is an essential amino acid in all fish species studied, and amino acid supplementation has been proven to promote the growth and feed utilization of fish, while the demand for arginine varies from 1% to 3.1% (3.8% to 8.1% of feed protein) by species [5]. Arginine is involved not only in protein synthesis but also in urea, glutamate, and other metabolic pathways. Protein synthesis is a key component involved in the growth reaction process and is limited by translation initiation [6–8]. TOR initiates translation and stimulates protein synthesis through S6K1 [9]. The TOR signalling pathway has become a hotspot in mammalian research, and dietary arginine supplementation has been shown to increase TOR signalling activity [1, 10]. Previous studies have shown that arginine can regulate TOR signalling pathways in *Carassius auratus* and *Cyprinus carpio* var. Jian [11, 12]. Therefore, the mechanism of the TOR signalling pathway in aquatic animals needs to be further researched [13].

The intestine is an important nutritional and immune organ of aquatic animals, and it plays a core role in the growth of fish [14]. However, it is vulnerable to attack by antigens, microorganisms, food toxic elements, and other foreign objects and is in close contact with the external environment [15]. Therefore, the health of the gut is critical to fish growth. In recent years, nutrients have been proven to play an important role in improving fish immunity [16, 17]. It has been shown that unbalanced dietary amino acids (insufficient or excessive) can reduce nutrient utilization and have a direct negative impact on immune organs and reactions [18–20]. At the same time, optimal arginine levels have positive effects on immune responses and the survival rates of *Penaeus monodon* [21] and *Ictalurus punctatus* [22]. It has been reported that optimal arginine levels can regulate pro- and anti-inflammatory cytokines [23, 24]. The NF-κB signalling pathway plays a key role in inflammation and is involved in the regulation of anti- and proinflammatory cytokines [25]. Proinflammatory cytokines contribute to the host's prevention mechanism of bacterial colonization or invasion [26, 27], and anti-inflammatory cytokines inhibit the overactivation of the immune response [28].

Oncorhynchus mykiss is the main cold water fish cultured in China and even globally. Compared with diploid, triploid *O. mykiss* has advantages of fast growth, low feed coefficient, and high meat content [29, 30]. The rapidly developing aquaculture industry also faces great challenges due to factors such as changes in the breeding environment, poor feed quality, diseases caused by bacteria and viruses, and the use of plant protein in feed [31, 32]. However, there have been few reports on the effects of arginine in a low-fishmeal diet on intestinal protein synthesis and inflammation-related genes of triploid *O. mykiss*. In this study, the effects of the dietary arginine level with low fishmeal on growth, digestion, intestinal protein synthesis, and expression of inflammatory factors as well as the influence of related genes were investigated to explore the causes of improvement in growth and digestion of trout due to arginine supplementation. This study is aimed at providing a theoretical basis for the optimization of triploid *O. mykiss* artificial compound feed.

Table 1: Ingredients and approximate composition of the test diets (air-dry basis, g/kg).

Items	Groups				
	G1	G2	G3	G4	G5
Soybean oil[a]	100.00	100.00	100.00	100.00	100.00
Fishmeal[a]	150.00	150.00	150.00	150.00	150.00
Fish oil[a]	62.80	62.80	62.80	62.80	62.80
Compound amino acids[b]	136.80	136.80	136.80	136.80	136.80
Soybean meal[a]	140.00	140.00	140.00	140.00	140.00
Dextrin	200.00	200.00	200.00	200.00	200.00
Gelatin	100.00	100.00	100.00	100.00	100.00
Beer yeast	50.00	50.00	50.00	50.00	50.00
Soybean phospholipid	20.00	20.00	20.00	20.00	20.00
$Ca(H_2PO_4)_2$	10.00	10.00	10.00	10.00	10.00
Microcrystalline cellulose	10.00	10.00	10.00	10.00	10.00
Vitamin premix[c]	3.00	3.00	3.00	3.00	3.00
Mineral premix[d]	2.00	2.00	2.00	2.00	2.00
Arginine[e]	0.00	2.50	5.00	7.50	10.00
Glycine[e]	10.00	7.50	5.00	2.50	0.00
Nutrient levels[f]					
Crude protein	415.40	413.10	412.60	414.10	413.60
Crude lipid	186.80	182.60	183.50	185.40	183.30
Ash	37.80	37.30	37.70	37.00	37.50
Moisture	82.00	81.80	85.60	86.00	81.30
Gross energy (MJ/kg)	21.73	21.45	21.48	21.62	21.50
Arginine (dry matter)	8.60	16.50	21.00	28.50	35.00
Arginine (protein)	20.70	39.70	50.60	68.60	84.30

[a]Dalong Feed Company, Harbin, China. [b]Composition of compound amino acids: arginine 6.2 g, lysine 21.3 g, phenylalanine 9.0 g, histidine 4.6 g, valine 17.2 g, leucine 20.1 g, isoleucine 14.6 g, cysteine 1.5 g, methionine 6.6 g, proline 8.6 g, aspartic acid 17.8 g, tyrosine 8.2 g, alanine 14.2 g, glutamic acid 36.3 g, and glycine 7.2 g. [c]The vitamin premix provided the following per kg of diets: VC 100 mg, VE 60 mg, VK_3 5 mg, VA 15000 IU, VD_3 3000 IU, VB_1 15 mg, VB_2 30 mg, VB_6 15 mg, VB_{12} 0.5 mg, nicotinic acid 175 mg, folic acid 5 mg, inositol 1000 mg, biotin 2.5 mg, and calcium pantothenate 50 mg. [d]The mineral premix provided the following per kg of diets: $MgSO_4 \cdot 7H_2O$ 2000 mg, KCl 1500 mg, $FeSO_4 \cdot 7H_2O$ 1000 mg, $CuSO_4 \cdot 5H_2O$ 20 mg, $MnSO_4 \cdot 4H_2O$ 100 mg, ZnSO4·$7H_2O$ 150 mg, KI 3 mg, NaCl 500 mg, $CoCl_2$ 5 mg, and Na_2SeO_3 3 mg. [e]Sigma Chemical Co., USA. [f]Nutrient levels were determined values.

2. Materials and Methods

2.1. Diets. The experimental diet formula and approximate composition are shown in Tables 1 and 2. Dextrin was used as a carbohydrate source. Fishmeal, soybean meal, and gelatin were used as sources of protein. Fish oil, soybean oil, and lecithin were used as sources of dietary lipids. Different levels of L-arginine (purity 99%, Sigma) were added: 0.86% (G1), 1.65% (G2), 2.10% (G3), 2.85% (G4), and 3.50% (G5). After weighing the ingredients according to the specified formula, they were homogenized in a blender. Ingredients were finely ground before mixing (<250 μm) and then blended with minerals and vitamins. After adding the lipid source, all ingredients were thoroughly mixed for 25 min. Distilled water was then added to achieve the right pellet

Table 2: Amino acid composition of the basal diets (air-dry basis, %).

Amino acids	G1	G2	G3	G4	G5
Dispensable amino acids					
Asp	4.43	4.18	4.43	4.22	4.20
Thr	1.87	1.79	1.69	1.68	1.64
Ser	2.23	2.06	2.47	2.45	2.40
Glu	6.51	6.38	6.56	6.54	6.49
Gly	2.66	2.68	3.38	3.36	3.31
Ala	2.74	2.36	2.66	2.74	2.69
Cys	0.72	0.72	0.75	0.73	0.78
Indispensable amino acids					
Val	2.01	2.10	2.06	2.02	2.14
Met	0.85	0.94	0.86	0.86	0.97
Ile	2.04	1.92	1.90	1.98	1.92
Leu	3.96	3.85	3.87	3.76	3.86
Tyr	1.69	1.61	1.64	1.62	1.57
Phe	2.25	2.25	2.05	2.04	2.06
Lys	2.96	2.69	2.38	2.28	2.49
His	1.33	1.26	1.20	1.18	1.22
Arg	0.86	1.65	2.10	2.85	3.50
Pro	2.40	2.63	2.83	2.81	2.61
EAA	20.35	20.9	20.89	21.4	22.34
TAA	41.51	41.07	42.83	43.12	43.85

Abbreviations: Asp: aspartate; Thr: threonine; Ser: serine; Glu: glutamate; Gly: glycine; Ala: alanine; Cys: cysteine; Val: valine; Met: methionine; Ile: isoleucine; Leu: leucine; Tyr: tyrosine; Phe: phenylalanine; Lys: lysine; His: histidine; Arg: arginine; Pro: proline; EAA: essential amino acid; TAA: total amino acids.

consistency, the mixture was further homogenized, and a pelletizer was used to form 1 mm pellets. Pellets were dried until the moisture content decreased to about 10% in a ventilated oven at 45°C and were then stored at −20°C until use.

2.2. Feeding Trial. Triploid rainbow trout (*O. mykiss*) were obtained from the Bohai Coldwater Fish Experimental Station of Heilongjiang River Fisheries Research Institute, Mudanjiang, China. Before commencing the experiment, the fish were acclimated to experimental conditions for 2 weeks and fed a basal diet twice daily. After acclimation, tanks were randomly assigned to each of the five diet groups, and trout (average initial weight 10.42 ± 0.2 g) were randomly stocked into 15 tanks (500 L) at 30 fish per tank. The fish were divided into five groups: a control group (fish fed a basal diet supplemented with 0.86% arginine) and four experimental groups (fish fed a basal diet supplemented with 1.65%, 2.10%, 2.85%, or 3.50% arginine). Each group consisted of three experimental tanks of replicates for the duration of the feeding trial, which was carried out in an indoor aquarium with a controlled water circulation system. The feeding trial lasted eight weeks, during which time the fish were fed to apparent satiation with the test diets at 09:00 h and 17:00 h daily. The fish were counted and weighed every 2 weeks from the onset of the experimental trial to check the body weight and adjust the amount of feed. During the experimental period, dissolved oxygen exceeded 6.0 mg/L, the water temperature was set as 15.0 ± 0.5°C, and the pH value was 6.8 ± 0.3. The NO_2-N and NH_4^+-N concentrations were no more than 0.02 and 0.5 mg/L, respectively. At the same time, one-third of the water was renewed per day to ensure water quality, and the feeding trials were conducted each day to ensure water quality.

2.3. Sample Collection. At the end of the experiment, fish were starved for 24 h to evacuate the alimentary tract contents prior to sampling. Nine fish per tank randomly selected from each treatment group were anesthetized with tricaine methanesulphonate MS-222 (75 mg/L). Blood samples were obtained from the caudal vein and then centrifuged at $5000 \times g$ at 4°C for 10 min to prepare the plasma. The plasma was stored at -80°C for subsequent plasma biochemical measurement. The intestine of three fish carp samples was quickly removed from the sampling site and the liver, the mid intestine (MI), was selected between pyloric and hindgut. The MI of six fish per tank was preserved and stored at -40°C for biochemical analysis. Meanwhile, the sampled three fish were dissected, and then, the proximal intestine samples were collected, immediately frozen in liquid nitrogen, and stored at -80°C for subsequent assay of gene expression. After that, three fish from each treatment group were taken out of the intestine to remove the contents after displacing and placed in a 4% formalin buffer solution for intestinal histological observation. At last, all fish were weighed to calculate the weight gain.

2.3.1. Nutrient Content. Experimental diets and fish were analyzed using the protocols based on the AOAC. The moisture content was determined by drying the sample in an oven at 105°C until a constant weight was obtained. Crude protein ($N \times 6.25$) was analyzed by measuring nitrogen using the Kjeldahl (2300, FOSS, Sweden) method. Ash content was analyzed by incineration at 550°C for 4 h. Crude lipid was quantified by following the Soxhlet method (Extraction System-811, BUCHI, Switzerland).

2.3.2. Amino Acid Determination. The amino acid composition of the fish was determined by an automatic amino acid analyzer (Hitachi L-8900). First, 0.1 g of the freeze-dried fish sample was weighed (accurate to 0.1 mg) and placed in a 50 mL ampoule that was then marked. Furthermore, 10 mL of 6 mol/L hydrochloric acid was added; the tube was quickly sealed and placed in a thermostatic drying oven at 110 ± 1°C for hydrolysis for 22 h, and the ampoule was opened after cooling. Next, a 150 mL rotary evaporation flask was filled with ultraclean water, vacuumed in a rotary evaporator (60°C), and evaporated until dried. The evaporating bottle was then washed with 0.02 mol L^{-1} hydrochloric acid several times, and the washing solution was transferred to a 10 mL volumetric bottle. Finally, 0.02 mol L^{-1} hydrochloric acid was used as a steady solution, and the sample was hydrolyzed after full mixing. Then, 2–3 mL of sample hydrolysate and standard mixed amino acid solution was absorbed, filtered into an automatic sample bottle, and capped, and the amino acid content was detected by an automatic amino acid analyzer (L-8900, Hitachi, Japan).

2.3.3. Biochemical Analysis. Biochemical analysis assays were performed using commercially available kits following the manufacturer's protocols (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Catalase (CAT; A007-2-1) activity was determined by measuring the decrease in H_2O_2 concentration at 240 nm. The reaction mixture contained 50 mm of potassium phosphate buffer (pH 7.0) and 10.6 mM freshly prepared H_2O_2. Superoxide dismutase (SOD; A001-3-2) activity was measured spectrophotochemically by the ferricytochrome method using xanthine/xanthine oxidase as the source of superoxide radicals. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 0.1 mM xanthine, 0.013 mM cytochrome c, and 0.024 IU mL^{-1} xanthine oxidase. One activity unit was defined as the amount of enzyme necessary to produce 50% inhibition of the ferricytochrome c reduction rate measured at 550 nm. The lysozyme (LZM; A050-1-1) content was measured using turbidimetry. Lipid peroxidation was analyzed in terms of malondialdehyde (MDA; A003-1-2) equivalents using the thiobarbituric acid reaction.

Intestinal samples were homogenized in 10 volumes (w/v) of ice-cold saline and centrifuged at $6000 \times g$ for 20 min at 4°C. Subsequently, the supernatants were used for biochemical analysis using the lipase assay kit (LPS; A054-2-1) and the amylase assay kit (AMS; C016-1-1). Trypsin (A080-2-2) content was determined by the ultraviolet colorimetric method, amylase (AMS) activity was determined by the starch-iodine colorimetric method, lipase (LPS) content was determined by the colorimetric method, and histone content was determined by the Coomassie bright blue method.

2.3.4. Histological Examination. The midguts of four fish in each replicate were randomly fixed in Bouin's solution for 48 h, then washed with water several times to remove the fixative solution, and embedded with conventional paraffin immersion. The sections were sliced using a slicing machine (KD1508) to a thickness of 6 μm. The slices were successively decolorized with ethanol, stained with hematoxylin and eosin, and finally sealed with neutral resin. After observation with a microscope (Leica MD 4000B), more than 10 intestinal sections were observed in each group.

2.3.5. Real-Time Polymerase Chain Reaction (PCR) Analysis. Total RNA was extracted using a total RNA isolation system kit (TaKaRa, Dalian, China) according to the manufacturer's recommended protocol. The concentration of RNA was quantified with a spectrophotometer (NanoDrop 2000, Thermo Fisher, Germany). The RNA quality was determined by analyzing the RNA integrity through agarose gel electrophoresis and by confirming that the A260/A280 nm absorbance ratio was between 1.8 and 2.0. Subsequently, RNA was reverse transcribed into cDNA using a PrimeScript™ RT reagent kit (TaKaRa, Dalian, China) following the manufacturer's instructions. The obtained cDNA templates were then stored at −80°C for later use. Quantitative PCR (qPCR) was carried out on the LightCycler®480 thermal cycler (Roche, Germany) at a total volume of 10 mL with the LightCycler®480 SYBR Green I Master (Roche, Germany) following the manufacturer's protocol. All amplification reactions were run in triplicate. Before qPCR amplification, the specificity and efficiency of the primers for β-actin were detected by constructing a standard curve using a serial dilution of cDNA, and the standard equation and correlation coefficient for each primer pair were determined. The specific primers of the β-actin gene and target genes are listed in Table 3. The expression results were analyzed using the $2^{-\Delta\Delta CT}$ method after verification that the primers were amplified with an efficiency of approximately 100% according to Guo et al. [32].

2.4. Calculations and Statistical Analysis. The calculation formula for the growth performance index was as follows:

$$\begin{aligned}
\text{Weight gain rate (WGR ; \%)} &= 100 \times \frac{(W_t - W_0)}{W_0},\\
\text{Condition factor (CF ; \%)} &= 100 \times \frac{W_t}{L_t^3},\\
\text{Feed conversion ratio (FCR)} &= \frac{W_f}{(W_t - W_0)},\\
\text{Specific growth rate (SGR ; \%/d)} &= 100 \times \frac{(\ln W_t - \ln W_0)}{t},\\
\text{Protein efficiency ratio (PER)} &= \frac{(W_t - W_0)}{(W_f \times \text{feed protein content})},\\
\text{Survival rate, \%} &= 100 \times \frac{W_t}{W_0},\\
\text{Hepatosomatic index (HSI ; \%)} &= 100 \times \left(\frac{\text{liver weight(g)}}{\text{body weight(g)}}\right),\\
\text{Viscerosomatic index (VSI ; \%)} &= 100 \times \left(\frac{\text{viscera(g)}}{\text{body weight(g)}}\right),
\end{aligned} \tag{1}$$

where W_0 is the initial body weight of the fish (g), W_t is the final body weight of the fish (g), L_t is the final body length (cm), W_f is the feed intake (g), and t is the test days (D).

Statistical software SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA) was used to conduct a one-way analysis of variance and Duncan's multiple comparisons of the data. Data are expressed as the mean ± standard deviation, and $P < 0.05$ indicated significant differences in the data within the group. The optimal arginine requirement of the triploid *O. mykiss* was determined by quadratic regression analysis with the protein deposition rate as the evaluation index using the Origin 6.0 software.

3. Results

3.1. Effects of Dietary Arginine Levels on Growth Performance of O. mykiss. Table 4 shows that at the end of the experiment the SR was not significant ($P > 0.05$). The WGR, SGR, and PER after the first increase reduce trends were the highest in the G3 group compared with those in the control group; the difference was significant ($P < 0.05$).

Table 3: Real-time PCR primer sequences.

Genes	Primer sequences forward (5′—3′)	Primer sequences reverse (5′—3′)	Accession number	Annealing temp. (°C)	Primer efficiency (%)
β-Actin	F: GGACTTTGAGCAGGAGATGG	R: ATGATGGAGTTGTAGGTGGTCT	XM_044093545.1	61.40	99.65
TOR	F: TGCCTCATCCTCACTACCCA	R: AAGCCTTTCTCAGCCTCGTC	XM_020506200.2	61.40	92.00
S6K1	F: CCTCCTGACAACAACGAGCT	R: GTGGTTGACATCTGCCAGGA	XM_029674978.1	55.00	96.00
IGF-1	F: ACTGTGCCCCTGCAAGTCT	R: CTGTGCTGTCCTACGCTCTG	M81904	55.00	95.00
IL-1β	F: ACCAGCCTTGTCGTTGTG	R: GTTCTTCCACAGCACTCTCC	AB010701.1	57.10	96.00
TGF-β	F: ACTGTGCCCCTGCAAGTCT	R: CTGTGCTGTCCTACGCTCTG	X99303	55.90	96.00
IL-6	F: CCTCAATCAACCCTACTCCC	R: GACACCTCACCCAGAACACG	XM_020472300.2	62.30	95.00
IL-8	F: GAATGTCAGCAGCCTTGTC	R: TCCAGACAAAYCYCCYGACCG	AJ310565.1	60.30	98.00
IL-10	F: CGACTTTAAATCTCCCATCGAC	R: GCATTGGACGATCTCTTTCTTC	AB118099.1	65.00	94.00
TNF-α	F: GGGGACAAACTGTGGACTGA	R: GAAGTTCTTGCCCTGCTCTG	AJ278085.1	58.40	93.00
NF-κB	F: CAGGACCGCAACATACTGGA	R: GCTGCTTCCTCTGTTGTTCCA	XM_031794907.1	58.40	96.00

β-actin gene as a housekeeping gene was used as an internal reference.

Table 4: Effects of dietary arginine level on growth performance of *O. mykiss* in various groups during the experiment.

Items	Groups					SE
	G1	G2	G3	G4	G5	
Initial body weight (g)	9.92 ± 0.17	9.99 ± 0.08	9.76 ± 0.08	9.83 ± 0.12	9.92 ± 0.10	0.05
Final body weight (g)	27.51 ± 2.11^{a}	28.89 ± 2.14^{ab}	29.58 ± 3.10^{ab}	31.70 ± 1.76^{b}	30.48 ± 2.88^{b}	1.07
WGR (%)	168.33 ± 6.12^{a}	166.15 ± 7.24^{a}	182.95 ± 6.84^{b}	178.14 ± 2.98^{ab}	178.35 ± 4.23^{ab}	3.48
Survival rate (%)	96.00 ± 2.31	92.00 ± 2.31	94.67 ± 3.53	94.67 ± 1.33	94.67 ± 3.53	1.09
FCR	1.24 ± 0.03^{b}	1.23 ± 0.10^{b}	1.06 ± 0.02^{a}	1.10 ± 0.03^{ab}	1.09 ± 0.02^{ab}	0.03
SGR (%/d)	1.71 ± 0.06^{a}	1.78 ± 0.04^{a}	1.82 ± 0.05^{b}	1.81 ± 0.02^{ab}	1.77 ± 0.02^{ab}	0.02
CF	1.16 ± 0.04^{b}	1.13 ± 0.05^{ab}	1.15 ± 0.02^{b}	1.11 ± 0.02^{a}	1.13 ± 0.03^{ab}	0.02
PER	1.47 ± 0.06^{a}	1.51 ± 0.06^{a}	1.69 ± 0.06^{b}	1.63 ± 0.04^{ab}	1.64 ± 0.03^{ab}	0.12

Note: values are presented as mean ± SE ($n = 3$). Values in the same column with different superscript letters are significantly different ($P < 0.05$). Abbreviations: WGR: weight gain rate; FCR: feed conversion; SGR: specific growth rate; PER: protein efficiency ratio; CF: condition factor.

The FCR showed a decreasing trend; the G3 group had the lowest, and the difference was significant ($P < 0.05$). The CF in the G1 and G3 groups was higher, and that in the G4 group was the lowest; there was a significant difference among all experimental groups ($P < 0.05$).

As shown in Figure 1, taking different arginine levels in the feed as the independent variable (x), quadratic regression analysis was conducted with an SGR. The regression equation between SGR and arginine level was as follows:

$$Y = -0.042X^2 + 0.208X + 1.561 \left(R^2 = 0.829\right) \quad (2)$$

When the arginine level was 2.48%, the SGR value of the triploid *O. mykiss* was the highest.

3.2. Effects of Dietary Arginine Levels on Body Parameters of O. mykiss. As shown in Table 5, there was no significant difference in the hepatosomal ratio among experimental groups ($P > 0.05$). The viscera ratio of the G1 group was the smallest, and there was a significant difference compared with the control group ($P < 0.05$).

3.3. Effects of Dietary Arginine Levels on the Composition of O. mykiss. As shown in Table 6, moisture levels in the G2, G4, and G5 groups were significantly lower than those in the control group (0%) when dietary arginine levels increased. The crude protein content first increased and then decreased when the dietary arginine level increased, and that in the G5 group was significantly lower than that in other groups ($P < 0.05$). The crude lipid and ash content showed no significant changes ($P < 0.05$).

3.4. Effects of Dietary Arginine Levels on the Amino Acid Profile of O. mykiss. As shown in Table 7, there was a significant difference compared with the control group with the increase of the level of arginine wherein the amino acid content in groups G4 and G5 increased significantly and that in group G5 was the highest.

3.5. Effects of Dietary Arginine Levels on Antioxidant Enzyme Activities of O. mykiss. As shown in Table 8, with the increase in the dietary arginine level, the activities of SOD in the serum and liver significantly increased ($P < 0.05$). The contents of MDA in the serum and liver decreased with an increase in the dietary arginine content ($P < 0.05$). When the arginine supplemental level was 2.10%, the content of MDA significantly decreased ($P < 0.05$). CAT activity was the highest in the G3 group, and the difference was

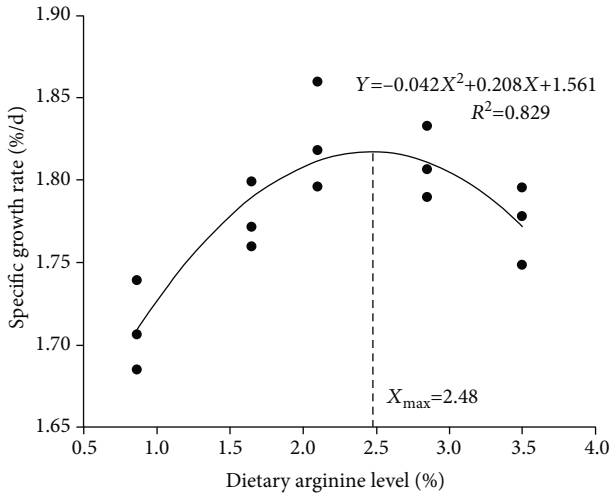

Figure 1: Regressive analysis between dietary arginine level and SGR of *O. mykiss*. *Note*: quadratic regression analysis relationship of specific growth rate to dietary arginine levels for *O. mykiss* fed with diets containing deficient (0.86%, 1.65%), optimum (2.10%, 2.85%), and excess (3.50%) arginine levels for 8 weeks, where "X" represents the dietary arginine level for the maximum specific growth rate of *O. mykiss*.

significant ($P < 0.05$). With the increase of the arginine supplemental level, the content of LZM first increased and then decreased. The maximum value was reached in the G4 group, and the difference was significant compared with other experimental groups ($P < 0.05$).

3.6. Effects of Dietary Arginine Levels on Intestinal Tissue Morphology of Triploid O. mykiss. As shown in Table 9, LPS content increased with the increase in the dietary arginine level among all groups, but LPS activity decreased with the increase in arginine content to 2.85%, and the difference was significant among all groups ($P < 0.05$). The AMS activity also significantly increased with the increase in arginine content ($P < 0.05$), and the highest AMS activity was found in the G5 group. There was no significant difference in trypsin activity among all groups ($P > 0.05$), but with the further increase in arginine content, trypsin activity tended to decrease.

3.7. Effects of Dietary Arginine Levels on Intestinal Tissue Morphology of Triploid O. mykiss. As shown in Table 10, villus height increased with an increase in the dietary arginine level ($P < 0.05$), but when the arginine content reached 2.85%, villus height decreased. Villus width and muscular thickness also first increased and then decreased with the increase in arginine content and reached the highest value in the 2.10% group, which was significantly different from that in the control group ($P < 0.05$).

In the arginine-deficient group, the tissue structure of the G1 and G2 groups (plates a, b, f, and g) was not complete, with tissue microvilli abruption, striatal source fusion, vague morphological outline, and edge absence (Figure 2). With the increase in the arginine level, the intestinal villi in the G3 group (plates c and h) abscissed and some striatal margins fused. The G4 and G5 groups (plates d, e, i, and j) had the optimum intestinal tissue morphology, showing well-developed villi, orderly arrangement, no fusion or exfoliation, increased goblet cell levels, and orderly arrangement of columnar epithelial cells.

3.8. Changes in Dietary Arginine Levels on Growth, Intestinal Inflammatory Factors, and Small Peptide Transporter Gene Expression Levels of Triploid O. mykiss. As shown in Figure 3, with an increase in the dietary arginine level, the expression level of TOR in the intestine first increased and then decreased and reached the highest value in the 2.86% group, which was significantly different from that in the control group ($P < 0.05$). The expression of S6K1 in the intestine was regulated by its upstream molecule TOR and reached the highest value in the 2.11% group, which was significantly higher than that in the control group ($P < 0.05$). Similarly, the expression level of IGF-1 in the intestinal tract first increased and then decreased and reached the highest value in the 2.11% group, and there was a significant difference among all groups ($P > 0.05$).

As shown in Figure 4, dietary supplementation with different levels of arginine had significant effects on the expression levels of proinflammatory cytokine (IL-1β, IL-6, IL-8, and TGF-α) genes in the intestine ($P < 0.05$). The expression levels of the IL-1β, IL-6, and IL-8 genes in the 3.5% and 2.85% groups were significantly lower than those in the control group ($P < 0.05$). TNF-α gene expression first decreased and then increased with the increase in the arginine supplemental level, and there were significant differences between the control and other groups ($P < 0.05$).

As shown in Figure 5, the expression of the TGF-β gene in the 2.85% group was the highest, and that in the 0.86% group was the lowest, with a significant difference ($P < 0.05$). IL-10 gene expression first decreased and then increased with the arginine supplemental level in a low-fishmeal diet and was the highest in the 2.85% group, with a significant difference ($P < 0.05$). The NF-κB gene expression first increased and then decreased with the arginine supplemental level and was the lowest in the 3.5% group with a significant difference ($P < 0.05$).

4. Discussion

4.1. Effects of Dietary Arginine Levels on Growth Performance of Triploid O. mykiss. A large number of studies have shown that arginine enhances the growth performance of fish [24, 33, 34]. In this study, the optimum proportion of arginine in the diet of juvenile triploid *O. mykiss* was 2.71% (6.52% of dietary protein), which was within the range of fish arginine requirements (3.8–8.1% of dietary protein) [35–37]. This result was similar to that of carp [11] (*C. carpio* var. Jian; 5.5% of dietary protein), juvenile grass carp (*Ctenopharyngodon idella*; 5.71% of dietary protein) [38], and blunt snout bream (*Megalobrama amblycephala*; 5.58% of dietary protein) [14]; higher than that of hybrid striped bass (*Morone chrysops* × *saxatilis*; 4.4% of dietary protein) [39], American redfish (*Sciaenops ocellatus*; 5.0% of dietary protein) [40], and Atlantic salmon (*Salmo salar*; 4.8% of

Table 5: Effects of dietary arginine level on HSI and VSI in *O. mykiss*.

Indices	Groups					SE
	G1	G2	G3	G4	G5	
HSI (%)	1.17 ± 0.04	1.15 ± 0.05	1.13 ± 0.04	1.14 ± 0.03	1.15 ± 0.03	0.02
VSI (%)	1.76 ± 0.05^{a}	1.88 ± 0.06^{b}	1.89 ± 0.06^{b}	1.92 ± 0.07^{b}	1.90 ± 0.05^{b}	0.04

Note: values are presented as mean ± SE ($n = 3$). Values in the same column with different superscript letters are significantly different ($P < 0.05$). Abbreviations: HSI: hepatosomatic index; VSI: viscerosomatic index.

Table 6: Effects of dietary arginine levels on body composition in *O. mykiss* in various groups.

Indices	Groups					SE
	G1	G2	G3	G4	G5	
Moisture	73.55 ± 0.35^{b}	71.76 ± 0.77^{a}	73.50 ± 0.48^{b}	73.09 ± 0.64^{ab}	72.80 ± 0.38^{ab}	0.25
Crude protein	12.36 ± 0.08^{a}	12.50 ± 0.19^{a}	13.03 ± 0.37^{b}	13.57 ± 0.07^{c}	13.23 ± 0.05^{bc}	0.12
Crude lipid	3.37 ± 0.17	3.66 ± 0.08	3.53 ± 0.21	3.64 ± 0.36	3.43 ± 0.17	0.11
Ash	1.73 ± 0.02	1.75 ± 0.03	1.75 ± 0.02	1.76 ± 0.05	1.74 ± 0.04	0.02

Note: values are presented as mean ± SE ($n = 3$). Values in the same column with different superscript letters are significantly different ($P < 0.05$).

Table 7: Effects of dietary arginine level on amino acid composition in whole fish of *O. mykiss* (DM basis).

Indices	Groups					SE
	G1	G2	G3	G4	G5	
EAA						
Thr	4.37 ± 0.21	3.73 ± 0.53	3.90 ± 0.05	3.97 ± 0.19	3.97 ± 0.23	0.32
Val	4.42 ± 0.26	4.44 ± 0.03	4.23 ± 0.05	4.52 ± 0.17	4.48 ± 0.08	0.03
Met	1.97 ± 0.63^{b}	1.39 ± 0.86^{ab}	0.81 ± 0.08^{a}	2.66 ± 0.04^{b}	2.70 ± 0.14^{ab}	0.04
Ile	3.42 ± 0.20^{ab}	3.31 ± 0.08^{ab}	3.12 ± 0.06^{a}	3.41 ± 0.22^{b}	3.39 ± 0.05^{b}	0.17
Leu	6.06 ± 0.65^{b}	5.65 ± 1.08^{ab}	5.21 ± 0.78	5.86 ± 0.54^{b}	5.63 ± 0.37^{ab}	0.36
Phe	3.58 ± 0.02^{c}	3.10 ± 0.01^{b}	3.08 ± 0.03^{ab}	3.10 ± 0.01^{b}	3.08 ± 0.03^{a}	0.02
Lys	3.45 ± 1.15^{a}	3.70 ± 1.43^{ab}	2.87 ± 0.14^{a}	5.44 ± 0.34^{b}	4.96 ± 0.28^{a}	0.13
His	2.20 ± 0.63	2.19 ± 0.56	2.56 ± 0.35	2.46 ± 0.36	2.49 ± 0.26	0.32
Arg	3.26 ± 0.09^{a}	3.92 ± 0.07^{ab}	3.65 ± 0.24^{a}	4.11 ± 0.32^{b}	4.16 ± 0.14^{b}	0.07
NEAA						
Asp	8.87 ± 0.53	7.55 ± 0.46	8.35 ± 0.32	8.01 ± 0.43	8.83 ± 0.26	0.27
Ser	3.68 ± 0.23	3.06 ± 0.84	3.37 ± 0.17	3.28 ± 0.53	3.47 ± 0.31	0.25
Glu	12.00 ± 0.48	10.66 ± 1.03	10.68 ± 0.26	11.34 ± 0.54	11.76 ± 0.59	0.43
Gly	3.32 ± 0.03	3.72 ± 0.46	3.34 ± 0.02	3.34 ± 0.16	3.33 ± 0.13	0.06
Ala	4.69 ± 0.37	4.59 ± 0.32	4.81 ± 0.25	4.73 ± 0.13	4.73 ± 0.15	0.24
Cys	0.94 ± 0.05	0.90 ± 0.04	0.87 ± 0.01	0.92 ± 0.05	0.97 ± 0.33	0.04
Tyr	2.28 ± 0.24	2.38 ± 0.07	2.25 ± 0.05	2.31 ± 0.09	2.26 ± 0.11	0.13
Pro	3.20 ± 0.14^{a}	4.31 ± 0.31^{ab}	4.91 ± 0.74^{b}	4.73 ± 0.62^{ab}	4.44 ± 0.38^{ab}	0.37
Total	73.25 ± 2.75^{ab}	72.02 ± 4.21^{a}	72.26 ± 2.47^{ab}	75.01 ± 2.45^{b}	75.29 ± 1.89^{ab}	2.21

Note: values are presented as mean ± SE ($n = 3$). Values in the same column with different superscript letters are significantly different ($P < 0.05$). Abbreviations: NEAA: nonessential amino acid.

dietary protein) [41]; and lower than that of grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂; 6.64–6.82% of dietary protein) [42] and black sea bream (*Sparus macrocephalus*; 7.74% of dietary protein) [43]. Some studies have shown that the arginine requirement of *O. mykiss* is 5.9% protein by growth [44], which is slightly lower than that of *O. mykiss* obtained in this study (6.52% of dietary protein). The different arginine requirements obtained from the above studies may be due to the species and size of the experimental fish, composition of base feed (including purified, semipurified, and practical feed), experimental design, culture conditions, etc. [42, 43]. For example, fish in this experiment were triploid *O. mykiss* and the feed was supplemented with 15% low fishmeal. In addition, the absorption

Table 8: Effects of dietary arginine on serum antioxidant capacity in *O. mykiss* in various groups.

Indices	Groups					SE
	G1	G2	G3	G4	G5	
SOD (U/mL)						
Serum	68.84 ± 4.23^{a}	75.86 ± 4.74^{b}	77.96 ± 2.02^{b}	89.33 ± 5.22^{c}	85.12 ± 4.16^{c}	3.23
Liver	7.98 ± 1.20^{a}	8.18 ± 1.43^{ab}	8.56 ± 1.08^{bc}	8.94 ± 1.12^{c}	8.35 ± 0.96^{b}	0.84
CAT (U/mL)						
Serum	9.88 ± 0.42^{a}	12.83 ± 0.78^{b}	14.66 ± 0.63^{c}	13.17 ± 0.58^{b}	13.10 ± 0.45^{b}	0.53
Liver	9.29 ± 0.76^{a}	10.55 ± 0.37^{b}	13.54 ± 0.63^{d}	11.90 ± 1.10^{c}	10.37 ± 0.62^{ab}	0.64
MDA (nmol/mL)						
Serum	7.85 ± 0.76^{b}	7.27 ± 0.64^{b}	5.43 ± 0.52^{a}	5.28 ± 0.45^{a}	7.12 ± 0.50^{b}	0.51
Liver	5.22 ± 0.98^{c}	4.48 ± 0.42^{b}	4.36 ± 2.83^{b}	4.33 ± 1.33^{b}	3.54 ± 0.91^{a}	0.15
LZM (U/mL)						
Serum	461.51 ± 9.34^{ab}	470.45 ± 10.76^{b}	486.25 ± 13.38^{b}	474.91 ± 10.31^{b}	426.46 ± 14.45^{a}	8.49
Liver	266.49 ± 9.97^{a}	276.12 ± 12.56^{a}	336.94 ± 11.21^{b}	361.51 ± 10.87^{b}	357.90 ± 14.48^{b}	7.93

Note: values are presented as mean ± SE ($n = 3$). Values in the same column with different superscript letters are significantly different ($P < 0.05$). Abbreviations: SOD: superoxide dismutase; MDA: malondialdehyde; CAT: catalase; LZM: lysozyme.

Table 9: Effects of dietary arginine on the intestinal digestive enzyme in *O. mykiss* in various groups.

Indices	Groups					SE
	G1	G2	G3	G4	G5	
LPS (U/gprot)	49.70 ± 3.38^{a}	72.09 ± 2.67^{b}	78.77 ± 3.54^{b}	99.75 ± 2.65^{c}	79.26 ± 3.86^{b}	3.01
Trypsin (U/mgprot)	715.15 ± 15.62	842.33 ± 14.62	918.60 ± 16.67	855.97 ± 18.56	772.09 ± 17.67	12.56
AMS (U/gprot)	125.77 ± 9.35^{a}	151.97 ± 12.47^{ab}	162.62 ± 10.88^{ab}	170.06 ± 11.11^{ab}	191.45 ± 8.42^{b}	7.47

Note: values are presented as mean ± SE ($n = 3$). Values in the same column with different superscript letters are significantly different ($P < 0.05$). Abbreviations: LPS: lipase; AMS: amylase.

Table 10: Effects of arginine supplementation on the intestinal morphology of triploid *O. mykiss* (μm).

Indices	Groups					SE
	G1	G2	G3	G4	G5	
Villus length	725.82 ± 12.42^{c}	738.76 ± 13.76^{d}	716.29 ± 11.87^{b}	706.56 ± 9.46^{a}	718.67 ± 10.42^{bc}	7.56
Villus width	334.71 ± 5.33^{ab}	332.07 ± 4.13^{ab}	331.29 ± 3.05^{a}	356.96 ± 2.85^{c}	348.17 ± 4.02^{b}	3.12
Muscular layer thickness	225.90 ± 3.02^{a}	232.98 ± 3.75^{b}	249.76 ± 4.56^{c}	238.52 ± 3.01^{bc}	240.32 ± 3.87^{bc}	2.76

Note: values are presented as mean ± SE ($n = 3$). Values in the same column with different superscript letters are significantly different ($P < 0.05$).

rate and bioavailability efficiency of arginine can affect the requirements [44]. When dietary arginine reaches or exceeds 2.71%, the rate of increase in gain rate, SGR, and PER decreases. Similar phenomena have been reported for fish such as golden pompano (*Trachinotus ovatus*), Nile tilapia (*Oreochromis niloticus*), and cobia (*Rachycentron canadum*) [24, 33, 36]. A large number of studies on mammals have confirmed that a decrease in growth performance due to excess arginine level is caused by arginine-lysine antagonism, but it is controversial whether this phenomenon exists in fish. It has been found that with the increase in dietary arginine and lysine levels, the digestibility of *O. mykiss* increases and decreases, respectively. Some studies have shown that excess lysine has an inhibitory effect on the growth performance of *O. mykiss* when the amount of arginine is moderate [45]. However, other studies have shown that there is no arginine-lysine antagonism between Japanese flounder (*Paralichthys olivaceus*) and yellow catfish (*Pelteobagrus fulvidraco*) [46]. Thus, whether arginine-lysine antagonism exists in fish remains to be further studied.

In this study, different levels of arginine had significant effects on the condition factor of triploid *O. mykiss*, which was consistent with the results of golden pompano (*Trachinotus ovatus*) and grass carp (*Ctenopharyngodon idella*) [33, 36]. As the arginine level increased, the hepatosomatic ratio decreased, while the viscerosomatic ratio increased in all experimental groups. These phenomena may be attributed to the use of energy stored in the liver through the appropriate level in the diet to synthesize protein in large quantities, resulting in a decrease in liver mass and volume, which is consistent with the results of studies on California bass [37, 47].

4.2. Effects of Dietary Arginine Levels on Body Composition of Triploid O. mykiss. In the present study, there was a significant change in the contents of crude lipids and ash with the

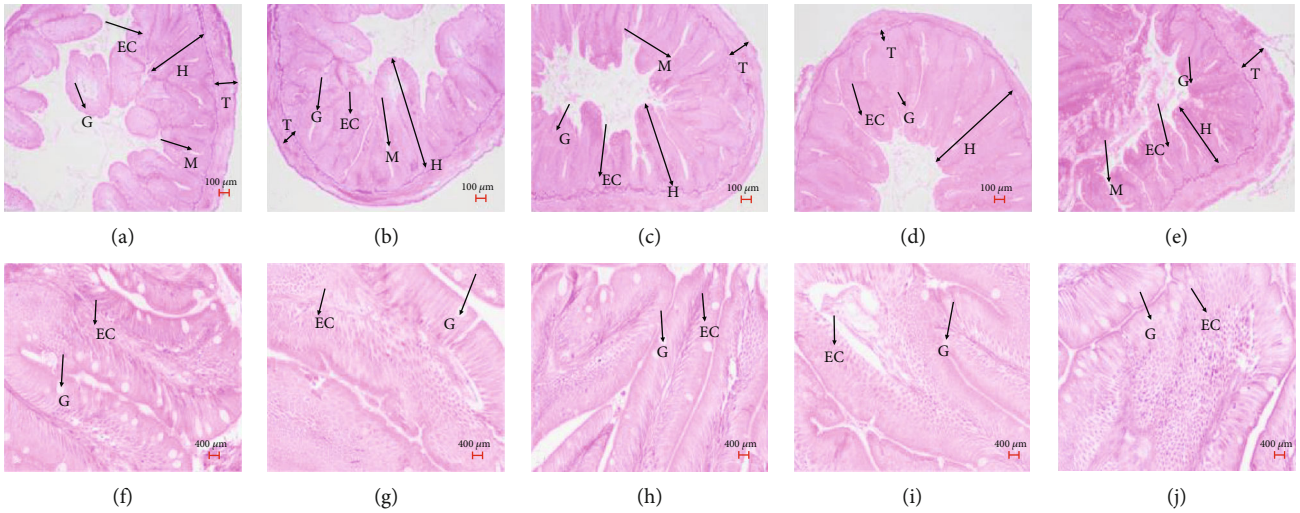

Figure 2: Effects of dietary arginine level on intestinal morphology of triploid *O. mykiss*. *Note*: (a) G1 arginine level in midgut tissue ×100; (b) G2 arginine level in midgut tissue ×100; (c) G3 arginine level in midgut tissue ×100; (d) G4 arginine level in midgut tissue ×100; (e) G5 arginine level in midgut tissue ×100; (f) G1 arginine level in midgut tissue ×400; (g) G2 arginine level in midgut tissue ×400; (h) G3 arginine level in midgut tissue ×400; (i) G4 arginine level in midgut tissue ×400; (j) G5 arginine level in midgut tissue ×400. M: microvilli; G: goblet cells; EC: epithelial cells; H: villi height; T: muscle layer thickness.

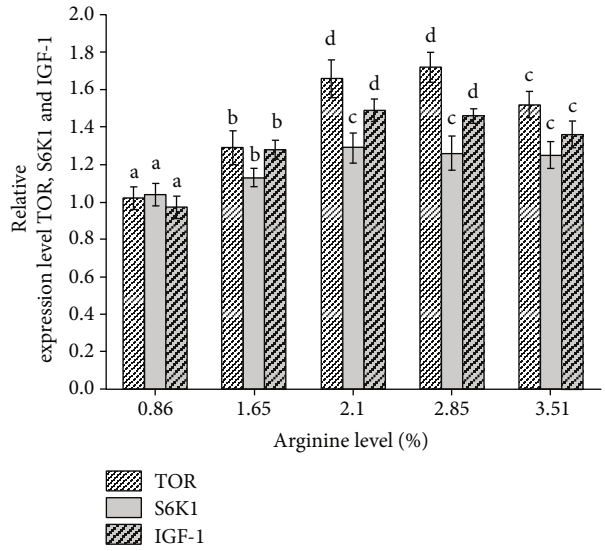

Figure 3: Effects of dietary arginine level on TOR, S6K1, and IGF-1 gene expression in triploid *O. mykiss*.

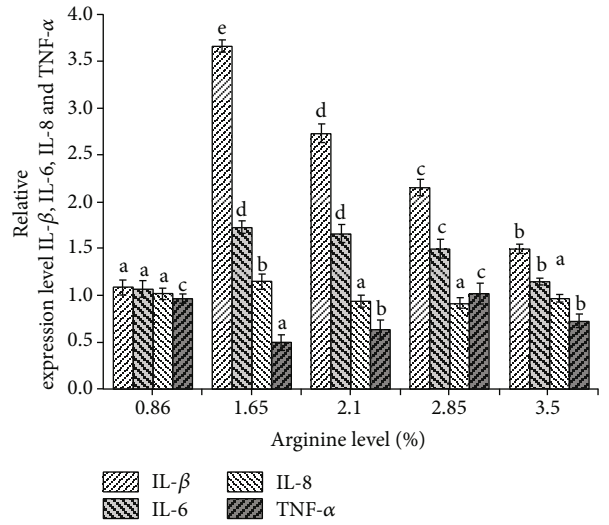

Figure 4: Effects of dietary arginine level on IL-1β, IL-6, IL-8, and TNF-α gene expression in triploid *O. mykiss*.

increase in arginine level, which was in agreement with the results of studies on yellow grouper (*Epinephelus awoara*) [47], Nile tilapia (*Oreochromis niloticus*) [48], and *M. amblycephala* [49]. The crude protein content showed a trend of first increasing and then decreasing with an increase in the arginine level. This indicates that arginine can be digested and absorbed by fish and deposited in the body, while excessive arginine may not be utilized and even have an inhibitory effect on production.

The deposition of organismal proteins by aquatic animals is achieved through the digestion and absorption of amino acids in the feed. Studies have reported that essential amino acid levels in the muscles of black sea bream increase with increased intake of dietary arginine [50]. In this study, an increase in the dietary arginine level increased the content of essential amino acids in the whole body, while it decreased the content of aspartic acid and proline. Moreover, the contents of valine, isoleucine, lysine, alanine, and tyrosine showed an increasing trend followed by a decrease. This indicates that when the amount of arginine consumed is optimal, many types of amino acids in the feed reach an ideal balance, and the amino acids digested and absorbed by fish are more involved in protein synthesis and deposition. However, when the arginine level in the feed exceeds the optimum level, redundant amino acids are oxidized and decomposed, causing losses and even damage to the organism. This indicates that intake of one type of amino acid may alter other amino acid contents, and the limitation of essential amino acids in the feed may increase the

nourishment of other essential or nonessential amino acids to achieve a balance of amino acids in the diet [11, 51].

4.3. Effects of Dietary Arginine Levels on Serum and Liver Antioxidant Indices of Triploid O. mykiss. In fish, SOD and MDA are widely recognized as important indexes of biological antioxidation and membrane lipid peroxidation, respectively [52, 53]. In this study, the optimal arginine level significantly reduced the serum and liver MDA levels, indicating that the optimal arginine levels were protective against oxidative damage in *O. mykiss*. The results of the study on grass carp showed that the highest MDA levels were found in the arginine-deficient group, and MDA content decreased and reached optimal levels when arginine content increased, indicating that arginine could protect the body by inhibiting oxidative damage. Superoxide and hydroxyl radicals are the main free radicals that strongly participate in oxidative damage. The results of this study indicate that the highest activity of SOD and CAT in serum and the liver with 3.5% dietary arginine contributes to the antioxidant capacity, as both antioxidant enzymes play an important role in the immune system of fish. It has been reported that high levels of antioxidant enzyme activity can inhibit oxidative stress in *Cynoglossus semilaevis* [54]. This result is similar to that of other studies wherein the optimal arginine level increased the antioxidant capacity of gills and muscles in Jian carp and grass carp [55, 56]. Different from our study, the increase in dietary arginine level reduced the activities of SOD, GPX, and CAT in the serum of yellow catfish [57], but the results of MDA were similar to ours. This distinction may be attributed to differences in the role of arginine levels, depending on different culture cycles and fish species.

4.4. Effects of Dietary Arginine Levels on Digestive Physiology of O. mykiss. Many factors affect the digestive enzyme activities of fish, such as feeding habits, growth stage, physicochemical factors (including water temperature, pH, and salinity), nutrition, and feed (including feed raw materials and additives) [51]. A study on Jian carp found that dietary arginine significantly increased the activities of protease and LPS, with no significant effect on AMS [11]. Studies on yellow catfish have shown that the arginine level has no significant effect on intestinal LPS, protease, and AMS, but AMS and protease have shown a trend of elevated variation [58]. Similar results have been found in juvenile black sea bream (*Acanthopagrus schlegelii*), where exogenous arginine promoted the activity of pepsin in the fish and AMS activity in the foregut and stomach, thus improving the digestion and absorption of nutrients in the intestines [52]. Similarly, this study showed that the addition of arginine to the low-fishmeal diet had a significant effect on both AMS and LPS contents but no significant effect on trypsin content, which showed a trend of first increasing and then decreasing. This might be because a certain amount of arginine in the diet cannot affect the activity of trypsin but can improve the activities of AMS and LPS.

The addition of exogenous arginine to the feed can promote the growth of fish. On the one hand, because arginine

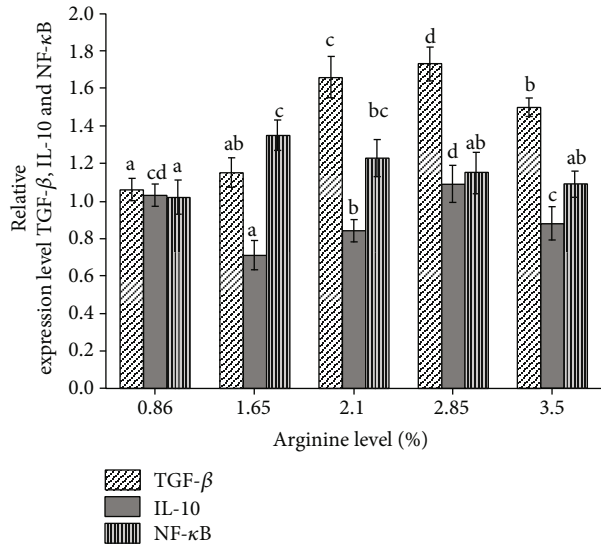

Figure 5: Effects of dietary arginine level on TGF-β, IL-10, and NF-κB gene expression in triploid *O. mykiss*.

can improve the activities of digestive enzymes in the intestines and stomach, it improves the digestion and absorption rate of the feed. On the other hand, arginine improves the intestinal morphology of fish to promote digestion. Studies on the foregut, midgut, and hindgut of hybrid striped bass showed that the arginine level significantly increased the villus and fold heights [39]. Similar results were found in a study on hybrid grouper (*E. fuscoguttatus* ♀ × *E. lanceolatus* ♂), where the addition of appropriate levels of arginine to the diet significantly increased the villi width, muscle thickness, and crease height in the foregut as well as crease height in the midgut of juvenile fish [42]. Arginine plays an important role in promoting the digestion and absorption of nutrients, protecting intestinal morphology, and preventing the invasion of pathogens. This study also showed that with the increase in the arginine level, villus and striate margin heights increased significantly. The catabolite of arginine was glutamine, which has the effect of maintaining intestinal immunity, preventing intestinal villus atrophy, and improving intestinal health [59]. The intestinal mucosa requires glutamine to support metabolic activities and maintain the integrity of the intestinal tract. The increase in glutamine content can promote the increase in the functional surface area of the intestinal mucosa, increasing the contact area between the intestinal tract and nutrients and enhancing the digestion and absorption of nutrients. Previous studies have demonstrated that glutamine increases the villus height of carp and promotes intestinal development [33]. Therefore, the development of intestinal morphology in juvenile *O. mykiss* is closely related to arginine level.

4.5. Effects of Arginine Levels on Growth and Intestinal Protein-Related Gene Expression of Triploid O. mykiss. Arginine is a stimulant of growth hormones IGF-1 and glucagon and is a precursor of polyamine synthesis; therefore, it is involved in growth-related processes in fish [60]. *O. mykiss* with 2.10% arginine had a higher rate of weight gain than

those with other proportions of arginine, which was attributed to the higher protein efficiency and IGF-1 gene expression in rainbow trout with a 2.10% arginine level. IGF-1 is an upstream signalling molecule that activates the protein synthesis signalling pathway in the body. Protein metabolism in the body is in a state of dynamic equilibrium. When the decomposition rate is lower than the synthesis rate, protein deposition is promoted, which manifests as fish body growth. Moreover, when protein synthesis is restricted by translation initiation, TOR stimulates protein synthesis by activating downstream S6K1 and 4E-BPS translation [10, 61]. In this study, the mRNA expression of TOR in the *O. mykiss* liver showed the same trend as that of IGF-1, suggesting that arginine may stimulate the secretion of IGF-1 and activate the TOR pathway to promote the synthesis of body proteins. The trends of mRNA expression changes of hepatic IGF-1 and TOR in *E. fuscoguttatus* (*E. fuscoguttatus* ♀ × *E. lanceolatus* ♂) were consistent with the results of the present study [62]. IGF-1 also activates TOR expression in the primary muscle cells of *Sparus aurata*, which verifies the results of this study at the cellular level [63]. Furthermore, the results of this study suggest that an appropriate level of arginine could significantly promote the expression of hepatic IGF-1, but excessive amounts of arginine disrupt the expression of hepatic IGF-1. This is possible because excessive arginine levels cause insulin resistance through a negative feedback system, leading to serine/threonine phosphorylation, as in higher animals, which may affect the expression of the IGF-1 gene [8, 14]. In the absence or with an excess of arginine, the expression levels of TOR and S6K6 in animals are reduced, leading to a decrease in protein biosynthesis and an increase in the proteolysis of cellular proteins. Therefore, the high protein and lipid contents of whole fish at a 2.10% arginine level may be due to the high expression of TOR and S6K6 in the liver [64].

In fish, a key component of the intestinal immune response is inflammation, which is mainly mediated by cytokines [65, 66]. IL-1β, IL-6, IL-8, and TNF-α are important proinflammatory cytokines [67, 68]. In this study, the expression of the IL-1β gene significantly increased in the arginine content-deficient group, while with increasing arginine levels, IL-1β was downregulated. This suggests that the arginine-deficient group induced intestinal inflammation by upregulating the expression of IL-1β in the intestine. Moreover, the expression of the intestinal TNF-α gene was increased. It is hypothesized that arginine may induce an increase in the nitric oxide (NO) levels of the *O. mykiss* intestine. In fish, arginine is a substrate for NO synthesis. Previous studies have shown that the addition of arginine promotes the production of NO in the muscles of grass carp, and optimal arginine levels significantly decrease the gene expression of IL-1β and TNF-α, suggesting that optimal arginine levels prevent or decrease the intestinal inflammatory response. Similar results have been found in Jian carp, wherein optimal arginine levels attenuated the inflammatory response in the intestine [69]. TGF-β and IL-10 are important anti-inflammatory cytokines that are mainly used to suppress the overactivation of the immune response [70, 71]. In this study, the optimal arginine level significantly improved the expression of TGF-β mRNA, indicating that it attenuated the inflammatory response [72, 73]. It was previously shown that the level of arginine upregulates the inflammatory factors TGF-β and TNF-α in the head kidney and enterocytes of carp. Arginine alleviates the increase in mRNA levels of lipopolysaccharide-induced TNF-α, IL-1β, and IL-6 by inhibiting the TLR4-MYD88 pathway, leading to improvement in the immune function of the body. NF-κB may play a key role in the inflammatory response [25]. In the present study, the optimal level of arginine significantly suppressed the expression of NF-κB, which was positively correlated with the expression of IL-1β, IL-6, IL-8, and TNF-α, inferring that the expression of proinflammatory factors may be realized by the downregulation of NF-κB. In addition, NF-κB expression was inhibited by anti-inflammatory cytokine gene expression. Thus, there was a negative correlation between anti-inflammatory cytokine gene expression and NF-κB expression, which was similar to the results obtained in grass carp gills [53].

5. Conclusion

The low-fishmeal diet (15%) supplemented with an appropriate level of arginine has positive effects on improving the growth, digestive enzyme activities, intestinal morphological structure development, and body immunity of juvenile triploid *O. mykiss*. Using specific growth rate as evaluation index, the optimal requirement of arginine for triploid *O. mykiss* was 2.48% by quadratic regression analysis. Based on the current research, the optimal arginine addition level can be further explored to replace a fishmeal with plant protein, to provide a theoretical basis for the optimization of artificial compound feed of triploid *O. mykiss*.

Data Availability

All datasets generated for this study are included in the article.

Ethical Approval

The protocols of animal care and treatment performed in the present study strictly complied with the Institutional Animal Care and Use Committee of the Ocean University of Shanghai.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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