

Research Article

Effects of Dietary Curcumin on Growth and Flesh Quality in Juvenile Genetically Improved Farmed Tilapia (GIFT, Oreochromis niloticus)

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This experiment was designed to investigate the effects of curcumin levels on growth, flesh quality, and oxidative resistance in juvenile genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*). Fish (initial mean weight: 4.5 ± 0.3 g) were randomly fed five diets with curcumin supplementation levels of 0 (control), 150, 300, 600, and 1,200 mg/kg. After 60 days of feeding, the hepatosomatic index was significantly reduced in the curcumin supplemented groups. The 300 mg/kg group had the highest crude protein content of the whole fish. Compared to the control group, the crude fat content of whole fish in the 150 and 300 mg/kg groups was significantly reduced by 18.83%-19.74%, respectively. The highest values for muscular hardness, chewiness, myofibrillar density, and proportion of small-sized myofibers ($<40 \,\mu$ m) were observed in the 300 mg/kg group. The levels of total nonessential amino acids and bitter amino acids in muscle were significantly lower in the 150 and 300 mg/kg groups than in the other groups. The 300 mg/kg group had higher levels of free sweet amino acids in the muscle than the other groups. Muscles from the 150, 300, and 600 mg/kg groups had significantly higher levels of umami amino acids than in the other groups. The levels of total free nonessential amino acids and total amino acids in serum were significantly higher in the 300 mg/kg group than in the control group. Serum aspartate transaminase activity was significantly lower in the curcumin supplementation groups than in the control group, and the serum alanine aminotransferase activity was significantly lower in the 150 mg/kg group than in the other groups. Serum superoxide dismutase activity was significantly higher in the curcumin supplementation groups than in the control group. Serum catalase activity was significantly higher in the 150 mg/kg group than in the other groups. The serum malondialdehyde level was lowest in the 150 mg/kg group. These results suggest that dietary curcumin supplementation in the diet at 150-300 mg/kg can effectively improve the nutritional value, muscle flavor, and antioxidant capacity of tilapia.

1. Introduction

Aquatic products are low in fat and high in protein, providing, on average, one-fifth of the total animal protein consumed by the world's population and are popular with consumers. However, due to the constant tension between the supply and demand of high-quality feed raw materials such as fish meal and fish oil, unconventional feed materials are used in large quantities, affecting the growth and quality of the aquaculture animals. And in recent years, with the continuous improvement of living standards and the imbalance of industrial development, aquatic products with poor flesh quality are difficult to meet the growing consumer demand of people. Flesh quality characteristics are affected by many factors, such as hardness, chewiness, myofibrillar density, myofibrillar diameter, and so on. Therefore, it is necessary to optimize aquaculture technology in aquatic research to improve the muscle quality and commercial value of aquatic products.

The formation of meat quality is largely related to the oxidation level of the body. The body's oxidation level depends on the amount or activity of antioxidants in the body. The

decrease in the level or activity of the body's antioxidants leads to the rapid oxidation of polyunsaturated fatty acid in the body, resulting in a large number of harmful substances such as oxygen free radicals and lipid peroxidation end products, resulting in excessive oxidation of the body and damage to the biofilm structure and function. The increased content of lipid peroxidation products in the body promotes the rapid conversion of oxymyoglobin (MbO₂) to metmyoglobin (Met Mb), which changes the color of meat from bright red to dark brown [1], ultimately leading to the oxidation and deterioration of muscle, shortening its shelf life and reducing its tenderness. Antioxidants in muscle (catalase (CAT), superoxide dismutase (SOD), etc.) can effectively inhibit the production and transfer of free radicals [2], reduce the degree of muscle oxidation, prevent the nutritional value of muscle, sensory quality, and tenderness reduction, thereby improving meat quality and extending the shelf life of meat products [3]. Studies have shown that adding antioxidants to the diets at the feeding stage can improve the antioxidant capacity of animals, reduce the rate of free radical oxidation in muscle, protect the integrity of biofilm structure and function in muscle, and achieve the goal of improving muscle mass [4]. Flesh quality has been shown to be improved by increasing the body's antioxidant capacity [5].

Curcumin is extracted from the rhizome of the Chinese herb Curcuma longa (C. longa L.). It is a crystalline, orange-yellow, acidic powder that has antioxidant properties, free radical scavenging, chelating, antitumor, detoxifying, anti-inflammatory, analgesic, and growth-promoting properties [6-9]. Curcumin can replace antibiotics in aquaculture and is widely used in clinical medicine to prevent and treat liver damage [10, 11]. Research on curcumin in the aquaculture sector is still in its early stages. Some studies have suggested that adding curcumin to the diet may benefit the liver, increase the activity of antioxidant enzymes, and aid in the growth in tilapia Oreochromis mossambicus [12], rainbow trout Oncorhynchus mykiss [13], and crucian carp Carassius auratus [14]. In a large number of experiments, the curcumin supplementation gradient was 0-800 mg/kg [15, 16], among which the optimal curcumin requirement of the snakehead fish (Channa argus) diet was 388.125 mg/kg [17]. However, while improving antioxidant capacity and immunity, there have been no reports of curcumin's effect on flesh quality in aquatic research.

Genetically improved farmed tilapia (GIFT, Oreochromis niloticus) was studied in this experiment. Tilapia has become one of the most important tilapia farming species due to its easy feeding, strong antistress ability, and delicious meat. However, due to the shortage of high-quality feed raw materials such as fish meal and fish oil, the diets of tilapia in captivity contain almost no animal ingredients, resulting in slow growth and low flesh quality. In recent years, to solve the negative problems caused by high-density farming [18] and antibiotic use and to realize the sustainable development of the tilapia farming industry [19, 20], great importance has been attached to the development and use of immunomodulators in recent years. However, while the focus has been on improving the body's antioxidant capacity and immunity, the effect of immune enhancers on flesh quality has been ignored. Since curcumin can significantly improve the antioxidant capacity of the body, it should also promote the improvement of muscle quality. Therefore, in this experiment, five different levels of curcumin powder were added to the diet. To investigate its effects on growth, flesh quality, and antioxidant enzyme activity in tilapia and to provide the scientific basis for its rational use in tilapia feed.

2. Materials and Methods

2.1. Experimental Diets. In the basal diet, the supplemental contents of soybean meal, fish meal, flour, corn gluten meal, soybean oil, corn oil, choline chloride, calcium dihydrogen phosphate, and premix were 8.40%, 61.00%, 18.00%, 3.00%, 3.00%, 3.00%, 0.10%, 2.50%, and 1.00%, respectively (Table 1). The actual contents of crude protein and crude fat in the basal diet were $44.05\% \pm 0.13\%$ and $12.60\% \pm 0.95\%$, respectively. The gradients of adding curcumin powder (provided by Hebei Handan Chenguang Biotechnology Group Co., Ltd.) to the basal diet were 0, 150, 300, 600, and 1,200 mg/kg. The raw materials were pulverized and sieved (<0.3 mm). The sifted raw materials and curcumin powder are accurately weighed and thoroughly mixed according to the feed formula. It is extruded in a twin-screw extruder $(32 \times 2, \text{ self-innovation})$ into a 2-mm diameter strip. After natural air drying, it is crushed into particles and stored at -20° C.

2.2. Management of Breeding Experiments. Experimental tilapia were obtained from a farm in Huanggang City, Hubei Province, and transported to the Hubei Key Laboratory of Animal Nutrition and Feed Science. After disinfection with povidone iodine, the experimental fish were placed in indoor temporary breeding barrels $(1.50 \times 1.50 \times 1.50 \text{ m})$ and fed the control diet for domestication for 1 week. A total of 450 healthy and well-sized fish with an initial body weight of $(4.5 \pm 0.3 \text{ g})$ were randomly divided into five groups with three replicates per group and 30 fish per replicate. The fish were fed with five different diets in 15 closed circular water cylindrical breeding barrels (diameter 60.0, height 80.0 cm). According to the principle of satiation, the fish were fed twice a day (8:00 and 16:00), and the feeding amount was adjusted according to the change in fish body mass. The water temperature, feeding, and death of the experimental fish were observed and recorded daily. During feeding, the light, the water temperature, the pH, the dissolved oxygen, the ammonia nitrogen salt, and the nitrite were natural light 28 \pm 0.5°C, 7.50-7.70, above 6.0, below 0.1, and below 0.01 mg/L, respectively. The feeding trial lasted for 60 days.

2.3. Sampling. At the end of the culture experiment, the fish were starved for 24 hr, and the number, total mass, and body length of the experimental fish in each barrel were recorded. Weight gain rate (WGR), specific growth rate (SGR), survival rate (SR), and feed conversion ratio (FCR) were calculated. Five fish from each barrel were randomly selected for proximate composition analysis. Blood samples were collected from the tail vein of 15 randomly selected fish in each barrel and kept in a freezer at 4°C for 3 hr. The top layer of the serum was separated by centrifugation at $3,200 \times g$ to determine the amino acid content of serum, serum biochemical

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0	1	1 1		,			
T4	Dietary curcumin levels (mg/kg)						
Item	0	150	300	600	1,200		
Ingredients (%)							
Soybean meal	8.40	8.40	8.40	8.40	8.40		
Fish meal	61.00	61.00	61.00	61.00	61.00		
Flour	18.00	18.00	18.00	18.00	18.00		
Corn gluten meal	3.00	3.00	3.00	3.00	3.00		
Premix ^a	1.00	1.00	1.00	1.00	1.00		
Soybean oil	3.00	3.00	3.00	3.00	3.00		
Corn oil	3.00	3.00	3.00	3.00	3.00		
Choline chloride	0.10	0.10	0.10	0.10	0.10		
Calcium dihydrogen phosphate	2.50	2.50	2.50	2.50	2.50		
Total	100.00	100.00	100.00	100.00	100.00		
Curcumin (mg/kg)	0.00	150.00	300.00	600.00	1,200.00		
Nutrient's composition (%)							
Moisture	9.19	9.12	9.56	9.74	9.22		
Crude protein	43.89	43.96	44.02	44.15	44.21		
Crude lipid	12.53	12.49	12.76	12.57	12.61		
Ash	20.15	20.38	20.58	20.75	20.49		

TABLE 1: Ingredients and proximate composition of experimental diets (dry matter basis).

Note: ^aThe vitamin and mineral premix formula were taken from Yu et al. [21].

indices, and antioxidant index. The viscera and liver were separated and weighed to determine the condition factor (CF), viscerosomatic indices (VSI), and hepatosomatic index (HSI). Dorsal muscles $(1.0 \times 1.0 \times 1.0 \text{ cm})$ were removed to determine muscle texture indices. The dorsal muscles were placed in formaldehyde fixative and electron microscope fixative, respectively. The remaining muscles above the dorsal lateral line were stored at -40° C to determine free amino acid content in the muscle.

2.4. Analytical Methods

2.4.1. Proximate Composition Analysis. The proximate compositions of muscle and diets were detected by the standard methods [22]. The moisture contents of experimental diets and whole fish were determined by the constant temperature drying weight loss method at $105 \pm 2^{\circ}$ C. Crude protein content was determined by the Kjeldahl nitrogen determination method. Crude fat content was determined by the Soxhlet extraction method. The ash content was determined by the muffle furnace combustion method at 550°C.

2.4.2. Muscular Texture Properties Analysis. The prepared tilapia samples were boiled in boiling water for 1 min. A texture analyzer (model TVT-300XP, Beijing, China) and a cylindrical aluminum probe with a diameter of 50 mm were used to determine the texture parameters, including hardness, springiness, chewiness, cohesiveness, and gumminess. The measurement parameters are as follows: the pre- and post-test speeds are 2 and 5 mm/s, respectively, and the deformation is 50% of the muscle thickness. Each specimen was pressed twice for 30 s. The texture index was analyzed using a texture analyzer program (version 3.42, Perten Instruments Inc., Hägersten, Sweden). Specific methods are described in the study by Cheng et al. [23].

2.4.3. Analysis of Muscle Fiber Structure. The samples of muscle were dehydrated in ethanol, cleaned in xylene, embedded in paraffin, and sectioned. Cross-sections of muscle were stained with hematoxylin and eosin. Finally, the microscopic structure of the muscle was observed using a light microscope (Olympus BX53, Tokyo, Japan). Myofibrillar diameter and myofibrillar density were measured using Image-J Launcher software.

2.4.4. Analysis of Free Amino Acid Content. The content of free amino acids in muscle and serum was determined using an automatic amino acid analyzer (HITICHI L-8900, Tokyo, Japan). The muscle samples were pretreated as follows: the fresh muscle samples were mixed with three times the volume of 10% sulfanilamide salicylic acid, homogenized, centrifuged (13,000 rpm, 15 min), and then placed on the 0.22 μ m water phase filter membrane overnight, and filtered into the sample bottle for detection.

2.4.5. Serum Biochemical Index Analysis. Serum samples frozen at -80°C were thawed at 4°C. An automated biochemical analyzer (BX-3010, Sysmex Corporation, Tokyo, Japan) was used to measure the activity of alkaline phosphatase (ALP), aspartate transaminase (AST), alanine aminotransferase (ALT), the content of glucose (GLU), total cholesterol (T-CHO), total protein (TP), albumin (ALB), and triglyceride (TG) in serum. The reagents used were purchased from Sysmex Corporation (Tokyo, Japan).

2.4.6. Antioxidant Indices in Serum. Frozen samples were thawed at 4° C and then diluted with 0.9% normal saline at a ratio of 1:9. The antioxidant indices of the diluted serum were determined. The malondialdehyde (MDA) content was determined by the thiobarbituric acid (TBA) method, and the activities of CAT and SOD were determined by the

TABLE 2: The growth performance of tilapia after 60 days of feeding.

Indexes	Dietary curcumin levels (mg/kg)						
	0	150	300	600	1,200		
IBW ¹ (g)	4.49 ± 0.05	4.51 ± 0.03	4.49 ± 0.03	4.51 ± 0.05	4.51 ± 0.01		
FBW^2 (g)	86.10 ± 2.5	85.44 ± 4.35	88.89 ± 5.88	85.91 ± 6.51	87.74 ± 7.52		
WGR ³ (%)	1818.57 ± 73.97	1794.93 ± 105.85	1878.93 ± 143.52	1803.91 ± 161.99	1847.25 ± 171.5		
SGR ⁴ (%/day)	5.18 ± 0.07	5.16 ± 0.1	5.24 ± 0.13	5.16 ± 0.15	5.2 ± 0.16		
FCR ⁵	0.98 ± 0.05	0.88 ± 0.05	0.94 ± 0.06	0.92 ± 0.05	0.87 ± 0.04		
CF ⁶ (%)	3.61 ± 0.35	3.77 ± 0.32	3.39 ± 0.29	3.2 ± 0.2	3.14 ± 0.28		
VSI ⁷ (%)	$8.31\pm0.68^{\rm b}$	7.35 ± 0.68^a	8.1 ± 0.73^{ab}	8.54 ± 0.93^{b}	7.98 ± 0.49^{ab}		
HSI ⁸ (%)	$1.67\pm0.08^{\rm c}$	$1.32\pm0.06^{\rm b}$	$1.28\pm0.04^{\rm b}$	1.02 ± 0.06^a	1.01 ± 0.08^{a}		
SR ⁹ (%)	100.00	100.00	100.00	100.00	100.00		

Note: Different letters on the same line indicate a significant difference (P<0.05); the same below. IBW, initial mean weight; FBW, final mean weight; WGR, weight gain rate; SGR, specific growth rate; FCR, feed conversion ratio; CF, condition factor; VSI, viscerosomatic index; HIS, hepatosomatic index; SR, survival rate. ⁶CF (condition factor, %) = FBW/final mean body length³ × 100. The calculation methods of ¹IBW, ²FBW, ³WGR, ⁴SGR, ⁵FCR, ⁷VSI, ⁸HSI, and ⁹SR refer to Yang et al. [24].

TABLE 3: The whole body composition of tilapia after 60 days of feeding (wet mass).

Indexes	Dietary curcumin levels (mg/kg)					
	0	150	300	600	1,200	
Moisture (g/kg)	664.81 ± 11.90	684.62 ± 36.21	659.03 ± 3.91	675.26 ± 24.85	669.29 ± 57.43	
Crude protein (g/kg)	172.58 ± 1.22^a	$178.22\pm1.95^{\text{b}}$	$185.3\pm1.32^{\rm c}$	$175.91\pm1.69^{\rm b}$	171.21 ± 2.21^{a}	
Crude lipid (g/kg)	$114.46\pm3.63^{\rm c}$	91.87 ± 1.53^a	92.91 ± 1.81^a	106.81 ± 2.80^{b}	$107.34\pm1.42^{\rm b}$	
Crude ash (g/kg)	50.47 ± 2.12^a	50.7 ± 1.07^a	50.61 ± 0.88^a	54.96 ± 2.00^{b}	59.42 ± 1.61^{c}	

Note: ^{a,b,c}Different superscript letters on the same line indicate significant difference (P < 0.05).

ammonium molybdate method and the water-soluble tetrazolium salt 1 (WST-1) method, respectively. All kits are produced by Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The catalog numbers of the kits of MDA, SOD, and CAT are A003-1, A001-1, and A007-1-1, respectively.

2.5. Statistical Analysis. Statistical analysis was performed using SPSS 26.0 (IBM Corp. Released in 2019. IBM SPSS Statistics for Windows, version 26.0. Armonk, NY, USA: IBM Corp.). Normality and homoscedasticity were first confirmed, then the data were used for one-way analysis of variance, and Tukey's multiple comparison method was used to test the significance of differences. The results were expressed as mean \pm standard deviation ($X \pm$ SD), and P < 0.05 indicated significant differences.

3. Results

3.1. Growth Performance. Compared with the control group, the HSI was significantly lower in the supplemental curcumin groups (P < 0.05); the VSI was lowest in the 150 mg/kg group and significantly decreased by 11.55% (P < 0.05) (Table 2). Supplemental curcumin decreased the FCR to some extent, but it was not significantly different (P > 0.05) (Table 2). The values of WGR, SGR, CF, and SR were not significantly different among groups (P > 0.05) (Table 2).

3.2. The Whole Fish Composition. The crude protein content was significantly higher when the curcumin supplementation level was 150-600 mg/kg than the control group (P < 0.05)

(Table 3). The crude lipid content was significantly lower in the curcumin supplementation group (P < 0.05), and the crude fat content was significantly reduced by 19.74%–18.83% in the 150 and 300 mg/kg groups, respectively (P < 0.05) (Table 3). Ash content was significantly increased by 8.90%–17.73% in the 600 and 1,200 mg/kg groups, respectively, compared with the control group (P < 0.05) (Table 3). Dietary curcumin levels did not significantly affect the moisture content (P > 0.05) (Table 3).

3.3. Muscle Texture. The hardness, springiness, and resilience of muscle first increased and then decreased. The hardness reached the highest value in the 300 mg/kg group (Table 4). Springiness reached the highest value in the 600 mg/kg group, which was significantly 19.05% higher than in the control group (P<0.05) (Table 4). Resilience was highest in the 600 and 1,200 mg/kg groups. Chewiness reached the highest value in the 300 mg/kg group, which was significantly 19.65% higher than that in the control group (P<0.05) (Table 4). Gumminess showed a gradually increasing trend, reaching the highest value in the 1,200 mg/kg group (P<0.05) (Table 4). Muscle cohesiveness was not significantly affected by dietary curcumin intake (P>0.05) (Table 4).

3.4. Morphology of Myofiber. The curcumin supplementation groups had a denser distribution of myofibers and smaller gaps between myofibers (Figure 1). Myofibrillar density was significantly higher in the 300 mg/kg group than in the other groups (P<0.05) (Figure 1). Myofibrillar diameter <40 μ m was significantly increased in the 300 mg/kg group compared with the other groups (P<0.05) (Figure 1). However,

TABLE 4: The muscular texture properties of tilapia after 60 days of feeding.

Tu damas	Dietary curcumin levels (mg/kg)						
Indexes	0	150	300	600	1,200		
Hardness (gf)	$1,\!269.00 \pm 19.77^{\rm a}$	$1,\!274.00\pm27.81^{ab}$	$1,\!317.40\pm20.42^{\rm b}$	$1,\!305.60\pm 30.33^{ab}$	$1,295.00 \pm 22.03^{ab}$		
Springiness	$0.42\pm0.02^{\rm a}$	0.43 ± 0.03^a	0.46 ± 0.03^{ab}	$0.50\pm0.02^{\rm b}$	$0.48\pm0.03^{\rm b}$		
Cohesiveness	0.38 ± 0.03	0.37 ± 0.03	0.40 ± 0.02	0.42 ± 0.03	0.42 ± 0.04		
Gumminess (gf)	493.08 ± 67.12^{a}	461.50 ± 54.18^{a}	504.26 ± 25.76^{a}	557.35 ± 31.45^{ab}	609.14 ± 79.12^{b}		
Chewiness (gf)	205.63 ± 22.24^a	210.23 ± 11.82^a	255.91 ± 9.11^{b}	249.51 ± 10.25^{b}	251.02 ± 8.75^{b}		
Resilience	0.15 ± 0.02^{ab}	0.15 ± 0.02^a	0.16 ± 0.01^{ab}	$0.18\pm0.01^{\rm b}$	$0.18\pm0.02^{\rm b}$		

Note: ^{a,b,ab}Different superscript letters on the same line indicate significant difference (P < 0.05).



FIGURE 1: Effects of dietary curcumin levels on the morphology of myofibers of tilapia. L0, the amount of curcumin added, was 0 mg/kg; L1, the amount of curcumin added was 150 mg/kg; L2, the amount of curcumin added was 300 mg/kg; L3, the amount of curcumin added was 600 mg/kg; L4, the amount of curcumin added was 150 mg/kg. (a) Myofiber microstructure of cross sections. Magnification 200x, MF: myofiber, the different letters on the columns indicate a significant difference. (b) Myofiber diameter and density of tilapia, the different letters on the columns indicate a significant difference.

1		Dietary curcumin levels (mg/kg)						
Amino acids	0	150	300	600	1,200			
Essential amino	acids							
Arg	168.42 ± 11.23	152.09 ± 4.65	166.75 ± 12.15	166.89 ± 6.64	170.11 ± 3.54			
His	$98.02\pm4.05^{\rm b}$	86.46 ± 4.9^{ab}	76.5 ± 6.93^a	84.77 ± 4.57^a	85.94 ± 2.80^{ab}			
Ile	81.88 ± 7.89^{ab}	76.20 ± 4.51^{ab}	74.20 ± 0.34^a	$86.79 \pm 1.10^{\rm b}$	$87.23 \pm 4.76^{\text{b}}$			
Leu	249.06 ± 7.03^{c}	180.65 ± 3.50^{a}	215.64 ± 5.39^{b}	$257.46 \pm 6.14^{\rm c}$	262.14 ± 3.98^{c}			
Lys	324.45 ± 2.94^{a}	326.93 ± 3.47^{a}	376.81 ± 1.30^{c}	340.73 ± 4.20^{b}	341.34 ± 3.94^{b}			
Met	65.97 ± 3.76^{b}	55.4 ± 2.35^a	61.93 ± 3.09^{ab}	$63.15\pm0.57^{\rm b}$	63.38 ± 2.36^{b}			
Phe	106.76 ± 3.62^{a}	108.39 ± 4.67^a	130.20 ± 1.15^{c}	$119.10 \pm 2.33^{\rm b}$	104.62 ± 2.72^a			
Thr	92.75 ± 2.06^a	111.98 ± 2.33^{d}	106.62 ± 4.24^{cd}	$102.96 \pm 2.16^{\rm bc}$	94.99 ± 3.80^{ab}			
Val	273.57 ± 3.76^{b}	252.37 ± 1.45^a	250.37 ± 6.77^a	277.46 ± 2.26^{b}	279.48 ± 0.88^b			
Nonessential am	nino acids							
Ala	218.45 ± 1.04	216.07 ± 3.49	215.86 ± 2.86	216.49 ± 3.18	215.73 ± 2.30			
Asp	57.60 ± 2.44	56.10 ± 0.85	57.31 ± 3.54	53.04 ± 5.06	54.52 ± 3.10			
Gly	82.43 ± 2.32^a	82.98 ± 3.31^a	$94.04\pm3.07^{\rm c}$	84.60 ± 4.05^a	67.48 ± 1.37^{b}			
Glu	$144.17 \pm 0.40^{\rm a}$	164.83 ± 2.48^{b}	$165.54\pm4.20^{\mathrm{b}}$	$162.88\pm1.80^{\text{b}}$	148.71 ± 2.05^a			
Pro	74.89 ± 2.54^a	$92.01 \pm 1.47^{\rm b}$	$97.04 \pm 3.35^{\text{b}}$	75.54 ± 0.58^a	75.12 ± 1.65^a			
Ser	$72.36\pm2.07a^b$	$90.39 \pm 2.76^{\rm c}$	81.83 ± 5.88^{bc}	71.68 ± 3.91^a	72.87 ± 2.84^{ab}			
Tyr	75.63 ± 5.12^{ab}	76.47 ± 1.58^{ab}	83.30 ± 3.69^{b}	77.24 ± 0.70^{ab}	71.25 ± 4.75^a			
Σ EAA ¹	$1,\!460.87 \pm 25.75^{\rm b}$	$1,\!350.47 \pm 18.43^{a}$	$1,\!459.00\pm31.57^{\rm b}$	$1,\!499.30 \pm 11.36^{\rm b}$	$1,\!489.23 \pm 4.03^{\rm b}$			
Σ NEAA ²	725.52 ± 10.48^{ab}	778.85 ± 8.95^{c}	794.92 ± 11.98^{c}	741.47 ± 7.82^{b}	705.67 ± 0.86^{a}			
TAAs ³	$2,\!186.39\pm33.34^{ab}$	$2,\!129.32\pm27.37^{a}$	$2,\!253.91 \pm 39.69^{\rm b}$	$2,\!240.77\pm7.06^{\rm b}$	$2,\!194.91 \pm 4.88^{\rm ab}$			
UAA^4	201.76 ± 2.26^{a}	$220.93 \pm 1.69^{\text{b}}$	222.85 ± 6.20^{b}	215.93 ± 6.81^b	203.23 ± 3.03^a			
SAA ⁵	865.33 ± 1.52^{a}	920.36 ± 8.37^{c}	972.19 ± 4.18^d	892.00 ± 7.37^b	867.53 ± 4.80^{a}			
BAA^{6}	$936.91 \pm 25.44^{\rm b}$	803.16 ± 16.35^{a}	845.37 ± 28.50^{a}	$936.51 \pm 13.04^{\rm b}$	$948.28\pm2.90^{\rm b}$			

Note: ¹ Σ EAA, total essential amino acids; ² Σ NEAA, total nonessential amino acids; ³TAAs, total amino acids; ⁴UAA, umami amino acid; ⁵SAA, sweet amino acid; ⁶BAA, bitterness amino acid. ^{a,b,c,ab,bc,cd}Different superscript letters on the same line indicate significant difference (*P*<0.05).

myofibrillar diameters of 40–100 and >100 μ m in the 300 mg/kg group were not significantly different from the control group (P > 0.05) (Figure 1). In the 150 mg/kg group, myofibrillar diameter >100 μ m was significantly increased compared to the control group (P < 0.05) (Figure 1). However, myofibrillar diameters $40-100 \,\mu\text{m}$ and diameter $<40\,\mu\text{m}$ were significantly decreased in the 150 mg/kg group compared to the control group (P < 0.05) (Figure 1). In the 600 and 1,200 mg/kg groups, myofibrillar diameter >100 μ m was significantly increased, and myofibrillar diameter 40–100 μ m was significantly increased compared with the control group (P < 0.05) (Figure 1). However, the myofibrillar diameter $<40 \,\mu\text{m}$ in the 600 mg/kg group was not significantly different from the control group (P > 0.05) (Figure 1). The myofibrillar diameter $<40 \,\mu$ m in the 1,200 mg/kg group was significantly reduced compared to the control group (P<0.05) (Figure 1).

3.5. Muscular Free Amino Acid Profiles. The content of free Σ NEAA and umami amino acid (UAA) (Asp, Glu) reached the highest value in the 300 mg/kg group (P<0.05) (Table 5). The content of sweet amino acid (SAA) (Gly, Ala, Pro, Thr, Ser, Lys) in muscle reached the highest value in the 300 mg/kg group, which was significantly higher than that in other experimental groups (P<0.05) (Table 5). The content of

free Σ EAA and bitterness amino acid (BAA) (Arg, His, Ile, Val, Leu, Met) in muscle was the lowest in the 150 mg/kg group (*P*<0.05) (Table 5).

3.6. The Content of Free Amino Acids in the Serum. Serum Σ NEAA and TAAs levels initially increased first and then decreased with increasing dietary curcumin levels. The levels of Σ NEAA and TAAs were highest in the 300 mg/kg group (P<0.05) (Table 6). Curcumin unaffected serum Σ EAA levels (P>0.05) (Table 6).

3.7. Serum Biochemical Indices. Compared to the group, the serum AST activity was significantly decreased in the curcumin supplementation group (P < 0.05) (Table 7). The activity of ALT in the 150 mg/kg group was significantly lower than the control group (P < 0.05) (Table 7). The ALP activity reached the highest value in the 300 mg/kg group (Table 7). The level of TG was significantly decreased in the 1,200 mg/kg group (P < 0.05) (Table 7).

3.8. Antioxidant Capacity. Serum SOD activity was significantly higher in the 300, 600, and 1,200 mg/kg groups than in the control group (P < 0.05) (Table 8). The SOD activity reached the highest value in the 600 mg/kg group, which significantly increased by 11.02% compared to the control group (P < 0.05) (Table 8). CAT activity was significantly

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A	Dietary curcumin levels (mg/kg)							
Amino acids	0	150	300	600	1,200			
Essential amino a	acids							
Arg	$10.27\pm0.68^{\rm c}$	8.26 ± 0.62^{b}	6.39 ± 0.17^a	7.48 ± 0.20^{ab}	$7.63\pm0.18^{\rm b}$			
His	26.46 ± 0.54	25.78 ± 1.38	24.82 ± 1.39	25.77 ± 1.86	26.61 ± 0.68			
Ile	$4.83\pm0.28^{\rm b}$	3.71 ± 0.02^a	$4.56\pm0.24^{\rm b}$	$5.82\pm0.20^{\rm c}$	$5.44\pm0.17^{\rm c}$			
Leu	6.60 ± 0.38	6.77 ± 0.16	6.82 ± 0.27	6.54 ± 0.16	6.62 ± 0.39			
Lys	13.15 ± 0.04^a	$21.91 \pm 1.36^{\text{b}}$	$22.23 \pm 1.60^{\rm b}$	$19.00\pm1.76^{\rm b}$	$19.00\pm0.89^{\rm b}$			
Met	4.64 ± 0.36^{ab}	3.98 ± 0.32^a	4.54 ± 0.27^{ab}	5.36 ± 0.35^{bc}	5.56 ± 0.31^{c}			
Phe	4.23 ± 0.06^a	$5.50\pm0.24^{\rm bc}$	5.91 ± 0.24^{c}	$5.46\pm0.20b^{c}$	$5.05\pm0.45^{\rm b}$			
Thr	11.92 ± 0.26^a	$13.81\pm0.28^{\rm b}$	$13.34\pm0.17^{\rm b}$	$15.18\pm0.27^{\rm c}$	$16.01\pm0.55^{\rm c}$			
Val	$7.22\pm0.07^{\rm a}$	7.10 ± 0.15^a	7.42 ± 0.37^a	8.06 ± 0.30^{b}	$8.22\pm0.08^{\rm b}$			
Nonessential ami	no acids							
Ala	42.05 ± 0.83^a	42.71 ± 1.88^a	$56.33 \pm 4.82^{\mathrm{b}}$	$52.96\pm0.11^{\rm b}$	51.99 ± 0.81^{b}			
Asp	$8.03\pm0.42^{\rm a}$	13.39 ± 0.71^{c}	$11.79\pm0.49^{\rm b}$	$11.60\pm0.45^{\rm b}$	$11.35\pm0.45^{\rm b}$			
Gly	68.04 ± 0.36^a	69.31 ± 2.57^a	69.94 ± 0.89^a	$82.30 \pm 1.99^{\text{b}}$	81.49 ± 1.82^{b}			
Glu	37.11 ± 0.40^a	38.53 ± 0.74^{ab}	$41.46\pm0.33^{\rm c}$	$39.63 \pm 1.44^{\text{bc}}$	$39.57 \pm 0.23^{\rm bc}$			
Pro	7.87 ± 0.11^{a}	$15.70\pm0.14^{\rm b}$	$26.60\pm0.10^{\rm c}$	$16.46\pm0.16^{\rm b}$	$16.20\pm0.62^{\rm b}$			
Ser	$6.40\pm0.18^{\rm a}$	$9.64\pm0.15^{\rm b}$	$9.61\pm0.22^{\rm b}$	$9.94\pm0.01^{\rm b}$	$11.24\pm0.34^{\rm c}$			
Tyr	4.10 ± 0.03^a	$4.68\pm0.05^{\rm bc}$	$5.40\pm0.24^{\rm d}$	4.88 ± 0.09^{c}	4.41 ± 0.27^{ab}			
Σ EAA ¹	80.51 ± 15.06	88.24 ± 17.09	96.04 ± 2.36	90.09 ± 13.49	100.15 ± 1.43			
Σ NEAA ²	173.60 ± 0.31^{a}	$193.96\pm1.43^{\mathrm{b}}$	221.13 ± 4.83^{c}	217.76 ± 1.65^{c}	216.26 ± 2.60^{c}			
TAAs ³	254.11 ± 14.75^{a}	282.19 ± 17.35^{ab}	$317.17 \pm 6.13^{\circ}$	307.86 ± 15.13^{bc}	$316.41\pm4.00^{\rm c}$			

TABLE 6: Serum-free amino acid contents of tilapia after 60 days of feeding (μ g/mL).

Note: ${}^{1}\Sigma$ EAA, total essential amino acids; ${}^{2}\Sigma$ NEAA, total nonessential amino acids; ${}^{3}TAAs$, total amino acids. ${}^{a,b,c,ab,bc}D$ ifferent superscript letters on the same line indicate significant difference (P<0.05).

TABLE 7: The serum biochemical index of tilapia after 60 days of feeding	3.
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Indexes	Dietary curcumin levels (mg/kg)					
	0	150	300	600	1,200	
AST (U/L)	89.67 ± 7.64^{c}	50.33 ± 3.06^a	51.33 ± 2.08^a	$70.33\pm5.03^{\rm b}$	$69.67\pm5.86^{\rm b}$	
ALT (U/L)	$36.33\pm0.58^{\rm b}$	26.33 ± 2.08^a	$33.67\pm2.52^{\rm b}$	35.00 ± 2.00^{b}	33.33 ± 2.08^{b}	
ALP (U/L)	33.00 ± 2.65^{ab}	32.67 ± 0.58^{ab}	$36.67 \pm 1.53^{\text{b}}$	29.33 ± 2.31^a	33.00 ± 2.65^{ab}	
TG (mmol/L)	$0.73\pm0.07^{\rm b}$	$0.79\pm0.07^{\rm b}$	$1.06\pm0.10^{\rm c}$	$1.08\pm0.08^{\rm c}$	0.45 ± 0.02^a	
GLU (mmol/L)	$6.50\pm0.44^{\rm c}$	4.55 ± 0.36^a	5.56 ± 0.23^{abc}	5.21 ± 0.47^{ab}	6.17 ± 0.59^{bc}	

Note: AST, aspartate transaminase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; TG, triglyceride; GLU, glucose a,b,c,ab,bc,abc Different superscript letters on the same line indicate significant difference (P<0.05).

TABLE 8: Serum antioxidant indices of tilapia after 60 days of feeding.

Indexes	Dietary curcumin levels (mg/kg)					
	0	150	300	600	1,200	
MDA (nmol/mL)	$7.74\pm0.18^{\rm a}$	$5.39\pm0.32^{\rm b}$	$5.90\pm0.06^{\rm b}$	7.23 ± 0.24^{a}	7.41 ± 0.19^{a}	
CAT (U/mL)	1.32 ± 0.07^a	$2.29\pm0.24^{\rm b}$	$4.67\pm0.31^{\rm c}$	$2.62\pm0.20^{\rm b}$	$2.56\pm0.04^{\rm b}$	
SOD (U/mL)	56.45 ± 0.25^a	56.74 ± 0.25^a	60.35 ± 0.13^{b}	63.44 ± 0.34^{c}	59.92 ± 0.18^{b}	

Note: MDA, malondialdehyde; CAT, catalase; SOD, superoxide dismutase. ^{a,b,c}Different superscript letters on the same line indicate significant difference (P < 0.05).

higher in the curcumin supplementation group (P < 0.05), and CAT activity was highest in the 300 mg/kg group (Table 8). In the 150 and 300 mg/kg groups, MDA levels were significantly reduced by 30.36% and 23.77%, respectively, compared to the control group (P < 0.05) (Table 8).

4. Discussion

4.1. Effects of Dietary Curcumin Levels on the Growth Performance of Tilapia. Curcumin supplementation did not promote the growth in tilapia in this study. Similar results

were found in rainbow trout *O. mykiss* [25]. Nevertheless, curcumin improved the growth performance of Nile tilapia *O. niloticus* and common carp *Cyprinus carpio* [26–28]. The results of this experiment were supported by the research of Hong et al. [29], who found that curcumin improved the CF and FCR of the greater amberjack *Seriola dumerili*. These may be related to the small size of the fish (initial body mass), the frequency of feeding, and the rearing environment.

4.2. Effects of Dietary Curcumin Levels on Nutrient Composition of Tilapia. The addition of dietary curcumin promotes the accumulation of fish protein and reduces the deposition of fish fat [26, 27]. The serum and muscle-free amino acid content increased, and the crude protein content was significantly higher in the 300 mg/kg group in this experiment, indicating that curcumin can improve the absorption and conversion of dietary protein and can effectively promote the accumulation of tilapia protein. The protective effect of curcumin on the liver, a key organ of energy metabolism in the body, may be responsible for the significant improvement in fat utilization in the curcumin supplementation groups compared to the control group. The energy metabolism system of tilapia can be modified by the addition of the correct amount of curcumin, which also causes the organism to utilize lipids more frequently and promote protein synthesis. In this experiment, the VSI in each curcumin supplementation group and the HSI in the 150 mg/kg group were significantly lower. These results suggest that curcumin supplementation could reduce visceral and hepatic fat while maintaining the standard liver shape.

4.3. Effects of Dietary Curcumin Levels on Muscle Hardness of Tilapia. In aquatic animals, myofibers can also change the texture of the muscle: the greater the myofibrillar density, the smaller the myofibrillar diameter, and the smaller the myofibril-myofibril interval, the better the myofibrillar density [30]. Myofibrillar characteristics consist primarily of myofibrillar diameter and density, the latter of which is positively correlated with muscle hardness [31]. In this study, the 300 mg/kg group had a denser structure and lower diameter of myofibers. These results suggest that by increasing myofibrillar density and decreasing myofibrillar diameter, muscular hardness is increased. Consumers typically believe that the more sophisticated the aquatic product, the better the taste [32]. The 300 mg/kg group had much harder and more chewy muscles. These results suggest that curcumin may increase the hardness and chewability of muscle by increasing the density of myofibers, decreasing the diameter of myofibers, and decreasing the distance between myofibers. These results also suggest that the muscle quality of tilapia can be successfully improved by consuming the right amount of curcumin. According to previous studies, improving the body's immunity can improve muscle quality, and some studies have shown that drip loss is positively related to oxidation [33]. We speculate that the improvement in muscle quality of curcumin-supplemented tilapia may be due to the improvement in the body's antioxidant capacity, but the specific mechanism needs to be further investigated.

4.4. Effects of Dietary Curcumin Levels on Nutritional Value and Flavor of Tilapia. The amino acid content of food determines its nutritional value. The higher the amino acid content, the higher the nutritional value of the food and the higher the human nutrition [34]. This study showed that dietary curcumin had no significant effect on the content of TAAs and Σ EAA of free amino acids in the muscle of tilapia. This may be because dietary curcumin did not affect the four amino acid transporters in the intestine, with no subsequent effect on the mRNA expression level of neutral amino acid transporters subsequently [35]. Serum-free Σ EAA levels had no effect, indicating that the serum amino acid pool could not promote the deposition of amino acids and proteins in tilapia muscle.

Amino acids in muscle not only confer nutritional value to the muscle but also impart a distinct flavor to the muscle [36]. Flavor is one of the critical factors for consumers to evaluate meat quality [37]. Muscle flavor in aquatic animals is influenced by the type and amount of free amino acids [14, 38, 39]. In this study, the composition of free amino acids in tilapia muscle was analyzed. It was found that the addition of an appropriate amount of curcumin significantly increased the content of UAA and SAA and significantly decreased the content of BAA in the muscle. However, the flavor of the muscle was no longer significantly affected when the curcumin was too high. The results of this study need to be confirmed by further experimental studies, as the effect of curcumin on the muscle flavor in aquatic animals has not yet been documented.

4.5. Effects of Different Dietary Curcumin Levels on the Immunity of Tilapia. AST and ALT are aminotransferases in the process of amino acid metabolism, and these two enzymes are essential indicators of liver function [40]. These two enzymes are mainly found in liver cells. When the liver is abnormal or the liver cells are damaged, the permeability of the liver cell membrane will increase, and AST and ALT in the liver cells will enter the blood. Therefore, the increased activity of AST and ALT indicates degeneration, damage, and necrosis of the liver [41]. ALP activity is closely related to fish growth and metabolism. It plays a vital role in the absorption and utilization of nutrients and is an essential enzyme for maintaining the health of the body [42]. GLU is derived from hepatic glycogen breakdown and intestinal absorption and is the major energy source in the body. TG is an important lipid substance and its level reflects, to some extent, lipid absorption and hepatic fat metabolism in the body [43].

Studies showed that dietary curcumin supplementation at 393.67 mg/kg significantly reduced serum ALT and AST activity in grass carp *Ctenopharyngodon idella* [16]; curcumin could reduce AST and ALT activities in the serum of Nile tilapia (*O. niloticus*) [44, 45]. In line with the above results, in this experiment, the activity of ALT in the 150 mg/kg group was significantly lower than that in the other experimental groups, and the activity of AST in the curcumin supplementation group was significantly lower than that in the control group, showing that the correct dosage of curcumin improved liver function. This indicates that dietary curcumin does not increase the activity of serum AST and ALT, does not cause liver damage, and can maintain liver health. In this experiment, a small amount of curcumin added to the diet did not reduce serum TG levels. These results are in agreement with Amer et al. [27]. With increasing levels of dietary curcumin, serum TG trended to increase and then decrease. The possible reason was that the low level of curcumin promoted the fat metabolism in tilapia, accelerated fat degradation, and increased the body's cholesterol content, leading to an increase in serum TG levels. High levels of curcumin can reduce cholesterol intake or promote the metabolic pathway of bile acid synthesis of cholesterol in the liver, which has the effect of lowering blood lipids [46]. This is also the reason why the crude fat content of whole fish first decreases and then increases. There are few publications on curcumin's control of lipid metabolism, and the processes involved require much research. The 300 mg/kg group in this trial had the most significant level of ALP activity. In the 150 mg/kg group, blood glucose levels decreased dramatically. Consistent with the results of this experiment, a study showed that the blood glucose level of fish fed the curcumin-rich diet was significantly reduced, indicating that curcumin can promote glucose uptake and glycogen synthesis to reduce blood glucose levels [12].

4.6. Effects of Different Dietary Curcumin Levels on the Antioxidant Capacity of Tilapia. MDA is a lipid-free radical formed from oxygen-free radicals and unsaturated fatty acids, which induces lipid reactions. The resulting lipid peroxidation products disrupt cell membrane integrity and cause oxidative damage to tissues and cells. Therefore, MDA is an indicator of the level of oxidative stress in the organism [47-49]. SOD and CAT are two necessary antioxidant enzymes that are widely distributed in organisms and play an important role in scavenging free radicals [50]. SOD can catalyze the decomposition of superoxide free radicals and remove oxygen free radicals. It inhibits the production of inflammatory cytokines and oxidative stress-induced inflammation [51]. CAT can effectively eliminate H_2O_2 produced by SOD by scavenging oxygen-free radicals [52]. In Pengze crucian carp C. auratus var. Pengze, dietary supplementation of 50 mg/kg curcumin significantly increased the activities of serum SOD and CAT activity and significantly reduced the serum MDA content [53]. Dietary supplementation of 50-200, 30, 15-60, and 200-800 mg/kg curcumin significantly increased the activities of liver SOD and CAT in Nile tilapia [27, 42, 54, 55]. In this study, serum MDA levels were significantly reduced in the 150 and 300 mg/kg groups. Serum CAT activity was significantly higher in the 300 mg/kg group than in the other experimental groups. The activity of serum SOD was significantly higher in the 300 mg/kg group. These results indicate that dietary supplementation with curcumin can significantly increase the activities of antioxidant enzymes and significantly reduce the content of MDA, which is consistent with the results of previous research. The decrease in serum MDA content may be due to curcumin reducing the antioxidant effect of stress response in the tilapia sampling process because curcumin has antioxidant and immune functions and can improve the antioxidant capacity of the body, including SOD, CAT, etc., to improve the antistress ability of the body. However, the optimal supplementation of curcumin in the diet is different, which may be related to the fish size, feeding time, and feeding environment.

5. Conclusions

To better improve the flesh quality and antioxidant capacity of juvenile genetically improved farmed tilapia, it is recommended that the dietary curcumin supplementation level be 150–300 mg/kg.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

All authors followed all applicable international, national, and/or institutional guidelines for the care and use of animals.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Xinyuan Li, Lifei Wu, and Li Duan are equally the first authors.

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