Vitamin C Requirement in Juvenile Black Sea Bass (Centropristis striata) Postprint

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Abstract

This experiment aimed to investigate the vitamin C requirement of juvenile black sea bass (Centropristis striata). A single-factor experimental design was adopted, selecting 630 juvenile black sea bass with an average body weight of (88.34±24.1) g, which were randomly divided into 7 groups with 3 replicates per group and 30 fish per replicate. Each group was fed isonitrogenous and isoenergetic diets with vitamin C levels (measured values) of 10.97 (control), 74.03, 148.33, 213.64, 335.33, 618.88, and 910.98 mg/kg, respectively. The effects of dietary vitamin C content on growth performance, serum biochemical indices, related enzyme activities, vitamin C accumulation in various tissues, and muscle composition of juvenile black sea bass were examined. The experimental period lasted 8 weeks. The results showed: 1) The weight gain rate and specific growth rate of the 74.03 mg/kg group were significantly higher than those of the 10.97, 618.88, and 910.98 mg/kg groups ($P<0.05$), the feed conversion ratio of the 74.03 mg/kg group was significantly lower than that of the 10.97 and 910.98 mg/kg groups ($P<0.05$), the protein efficiency ratio of the 10.97 and 910.98 mg/kg groups was significantly lower than that of the 74.03 and 618.88 mg/kg groups ($P<0.05$), and the condition factor of the 335.33 mg/kg group was significantly lower than that of the 74.03, 148.33, and 213.64 mg/kg groups ($P<0.05$). 2) The serum total protein content of the 10.97 and 74.03 mg/kg groups was significantly lower than that of the other groups ($P<0.05$), the serum triglyceride content of the 618.88 and 910.98 mg/kg groups was significantly lower than that of the other groups ($P<0.05$), and the serum lysozyme activity of the 10.97 mg/kg group was significantly lower than that of the 213.64, 335.33, 618.88, and 910.98 mg/kg groups ($P<0.05$). 3) The serum superoxide dismutase (SOD) activity of the 335.33 mg/kg group was significantly higher than that of the 10.97, 74.03, and 910.98 mg/kg groups ($P<0.05$), the serum total antioxidant capacity (T-AOC) of the 335.33 mg/kg group was significantly higher than that of the 10.97, 618.88, and 910.98 mg/kg groups ($P<0.05$), and the serum malon-

dialdehyde (MDA) content of the 335.33 mg/kg group was significantly lower than that of the 10.97 mg/kg group (P<0.05). 4) The hepatic SOD and catalase (CAT) activities of the 10.97 mg/kg group were significantly lower than those of the other groups (P<0.05), and the hepatic MDA content of the 148.33, 335.33, and 910.98 mg/kg groups was significantly lower than that of the 10.97 mg/kg group (P<0.05). 5) The muscle SOD activity and T-AOC of the 74.03 mg/kg group were both significantly higher than those of the other groups (P<0.05). 6) The serum glutamic-pyruvic transaminase (GOT) activity of the 213.64, 335.33, 618.88, and 910.98 mg/kg groups was significantly lower than that of the 10.97 mg/kg group (P<0.05), the hepatic GOT activity of the 10.97 mg/kg group was significantly lower than that of the other groups (P<0.05). The serum glutamic-oxaloacetic transaminase (GPT) activity of the 10.97 and 74.03 mg/kg groups was significantly higher than that of the other groups (P<0.05). 7) The vitamin C accumulation in serum of the 910.98 mg/kg group was significantly higher than that of the other groups (P<0.05), the vitamin C accumulation in liver of the 910.98 mg/kg group was significantly higher than that of the 10.97, 74.03, 148.33, and 213.64 mg/kg groups (P<0.05), and the vitamin C accumulation in muscle of the 618.88 mg/kg group was significantly higher than that of the 10.97, 74.03, 148.33, and 213.64 mg/kg groups (P<0.05). 8) The muscle moisture content of the 74.03 mg/kg group was significantly lower than that of the 10.97, 148.33, and 213.64 mg/kg groups (P<0.05), and the muscle collagen content of the 910.98 mg/kg group was significantly higher than that of the other groups (P<0.05). In conclusion, using specific growth rate, hepatic vitamin C accumulation, and serum lysozyme activity as evaluation criteria, the dietary vitamin C requirements for juvenile black sea bass were determined to be 66.66, 309.93, and 345.11 mg/kg, respectively, through broken-line regression model analysis.

Full Text

Vitamin C Requirement of Juvenile Striped Bass (Centropristis striata)

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Abstract: This experiment investigated the dietary vitamin C requirement of juvenile striped bass (*Centropristis striata*). Using a single-factor experimental design, 630 juvenile striped bass with an average body weight of (88.34±24.1) g were randomly allocated into 7 groups with 3 replicates each (30 fish per replicate). The fish were fed isonitrogenous and isocaloric diets containing measured vitamin C levels of 10.97 (control), 74.03, 148.33, 213.64, 335.33, 618.88, and 910.98 mg/kg. The effects of dietary vitamin C on growth performance, serum biochemical parameters, enzyme activities, tissue vitamin C accumulation, and

muscle composition were evaluated over an 8-week feeding trial. The results showed: (1) Weight gain rate and specific growth rate in the 74.03 mg/kg group were significantly higher than those in the 10.97, 618.88, and 910.98 mg/kg groups ($P<0.05$), while feed conversion ratio was significantly lower than in the 10.97 and 910.98 mg/kg groups ($P<0.05$). Protein efficiency ratio in the 10.97 and 910.98 mg/kg groups was significantly lower than in the 74.03 and 618.88 mg/kg groups ($P<0.05$). Condition factor in the 335.33 mg/kg group was significantly lower than in the 74.03, 148.33, and 213.64 mg/kg groups ($P<0.05$). (2) Serum total protein content in the 10.97 and 74.03 mg/kg groups was significantly lower than in other groups ($P<0.05$). Serum triglyceride content in the 618.88 and 910.98 mg/kg groups was significantly lower than in other groups ($P<0.05$). Serum lysozyme activity in the 10.97 mg/kg group was significantly lower than in the 213.64, 335.33, 618.88, and 910.98 mg/kg groups ($P<0.05$). (3) Serum superoxide dismutase (SOD) activity in the 335.33 mg/kg group was significantly higher than in the 10.97, 74.03, and 910.98 mg/kg groups ($P<0.05$). Serum total antioxidant capacity (T-AOC) in the 335.33 mg/kg group was significantly higher than in the 10.97, 618.88, and 910.98 mg/kg groups ($P<0.05$). Serum malondialdehyde (MDA) content in the 335.33 mg/kg group was significantly lower than in the 10.97 mg/kg group ($P<0.05$). (4) Liver SOD and catalase (CAT) activities in the 10.97 mg/kg group were significantly lower than in all other groups ($P<0.05$). Liver MDA content in the 148.33, 335.33, and 910.98 mg/kg groups was significantly lower than in the 10.97 mg/kg group ($P<0.05$). (5) Muscle SOD activity and T-AOC in the 74.03 mg/kg group were significantly higher than in all other groups ($P<0.05$). (6) Serum glutamic oxaloacetic transaminase (GOT) activity in the 213.64, 335.33, 618.88, and 910.98 mg/kg groups was significantly lower than in the 10.97 mg/kg group ($P<0.05$), while liver GOT activity in the 10.97 mg/kg group was significantly lower than in all other groups ($P<0.05$). Serum glutamic-pyruvic transaminase (GPT) activity in the 10.97 and 74.03 mg/kg groups was significantly higher than in other groups ($P<0.05$). (7) Serum vitamin C accumulation in the 910.98 mg/kg group was significantly higher than in all other groups ($P<0.05$). Liver vitamin C accumulation in the 910.98 mg/kg group was significantly higher than in the 10.97, 74.03, 148.33, and 213.64 mg/kg groups ($P<0.05$). Muscle vitamin C accumulation in the 618.88 mg/kg group was significantly higher than in the 10.97, 74.03, 148.33, and 213.64 mg/kg groups ($P<0.05$). (8) Muscle moisture content in the 74.03 mg/kg group was significantly lower than in the 10.97, 148.33, and 213.64 mg/kg groups ($P<0.05$), while muscle collagen content in the 910.98 mg/kg group was significantly higher than in all other groups ($P<0.05$). Based on specific growth rate, liver vitamin C accumulation, and serum lysozyme activity as evaluation criteria, broken-line regression analysis indicated that the dietary vitamin C requirements for juvenile striped bass were 66.66, 309.93, and 345.11 mg/kg, respectively.

Keywords: *Centropristis striata*; vitamin C; growth; antioxidant; vitamin C accumulation

1.1 Experimental Diet Preparation

Seven isonitrogenous and isocaloric experimental diets were formulated using fish meal, casein, and soybean meal as protein sources, and fish oil and soybean oil as lipid sources. Vitamin C was supplemented as ascorbyl monophosphate (35% active content) at levels of 0 (control), 50, 150, 300, 600, 1,200, and 2,400 mg/kg (pure vitamin C equivalent, not ascorbyl phosphate). Dietary composition and nutrient levels are presented in Table 1 . Micro-ingredients were mixed using the progressive enlargement method. Soybean meal and choline chloride were ground to pass through an 80-mesh sieve. All ingredients were weighed according to formulation, mixed thoroughly in a blender, and processed into 5 mm diameter pellets using a pellet mill. The pellets were dried at 50 °C and stored at -20 °C until use. The actual vitamin C content of each diet was measured by high-performance liquid chromatography at Qingdao Kepu R&D Technology Center Ltd., yielding measured values of 10.97 (control), 74.03, 148.33, 213.64, 335.33, 618.88, and 910.98 mg/kg.

1.2 Experimental Design and Culture Management

The feeding trial was conducted at a mariculture farm in Rizhao. Prior to the experiment, juvenile fish were acclimated in 500 L circular PE tanks for two weeks, during which they were fed the basal diet to adapt to the experimental environment and feed. After acclimation, fish were fasted for 24 h, and 630 healthy one-year-old juvenile striped bass with uniform size, robust condition, and average body weight of (88.34±24.1) g were randomly distributed into 21 tanks (7 groups × 3 replicates). Each replicate contained 30 fish. No significant difference in initial body weight was observed among groups ($P>0.05$). During the 8-week trial, fish were hand-fed to satiation twice daily (07:00 and 17:00). Feed intake was recorded, and uneaten feed and feces were removed 30 minutes after feeding to estimate feed consumption.

The experiment employed a flow-through water system. Water temperature (18–24 °C), pH (6.8–7.3), dissolved oxygen (6.24–7.08 mg/L), salinity (22–24), and ammonia nitrogen (0.45–0.63 mg/L) were monitored regularly. Fish health was checked daily for signs of vitamin C deficiency.

1.3 Sample Collection

At the end of the trial, fish were fasted for 24 h before sampling. Fish in each tank were counted and weighed to calculate weight gain rate (WGR), specific growth rate (SGR), condition factor (CF), and survival rate (SR). Total feed consumption was recorded to determine feed conversion ratio (FCR) and protein efficiency ratio (PER). Five fish were randomly selected from each tank, anesthetized with MS-222, and blood samples were collected from the caudal vein. Blood was stored at 4 °C overnight, then centrifuged at 3,500 r/min for 10 min to obtain serum for analysis of biochemical parameters, enzyme activities, antioxidant indices, and vitamin C accumulation. Fish were then dissected on

ice to isolate viscera and liver for calculation of viscerosomatic index (VSI) and hepatosomatic index (HSI). Liver and dorsal muscle samples were collected for antioxidant analysis and vitamin C determination. Remaining dorsal muscle was stored at -20 °C for proximate composition analysis.

1.4.1 Growth Performance, Morphological Indices, and Feed Utilization Formulas

Weight gain rate (%) = 100 × [(final mean body weight - initial mean body weight) / initial mean body weight]
Specific growth rate (%/d) = 100 × (ln final mean weight - ln initial mean weight) / experimental days
Survival rate (%) = 100 × final fish number / initial fish number
Hepatosomatic index (%) = 100 × liver weight / body weight
Viscerosomatic index (%) = 100 × viscera weight / body weight
Condition factor (g/cm^3) = body weight / body length^3
Feed conversion ratio (FCR) = total dry feed intake / total body weight gain
Protein efficiency ratio (PER) = (final mean weight - initial mean weight) / total dietary protein intake

1.4.2 Serum Biochemistry and Tissue Antioxidant Indices

Serum total protein and alkaline phosphatase activity were determined by microplate enzyme assays. Serum triglyceride and cholesterol were measured using GPO-PAP and COD-PAP methods, respectively. Serum lysozyme activity was determined by turbidimetry. Superoxide dismutase (SOD) activity in serum, liver, and muscle was measured by the hydroxylamine method. Catalase (CAT) activity was determined by visible light spectrophotometry. Total antioxidant capacity (T-AOC) and vitamin C accumulation were measured by colorimetry. Glutamic oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), and malondialdehyde (MDA) were determined by microplate methods. All kits were purchased from Nanjing Jiancheng Bioengineering Institute, and assays were performed strictly according to manufacturer instructions.

1.4.3 Muscle Composition Analysis

Muscle samples were sent to Qingdao Kepu R&D Technology Center Ltd. for analysis. Crude protein, crude lipid, moisture, and ash were determined by Kjeldahl, Soxhlet, direct drying, and muffle furnace combustion (550 °C) methods, respectively. Collagen content was measured by the hydroxyproline method [12].

1.5 Statistical Analysis

Data were analyzed by one-way ANOVA using SPSS 17.0 software. Duncan' s multiple range test was used to compare differences among groups, with signifi-

cance set at $P<0.05$. Results are presented as mean ± standard deviation (SD). Graphs and regression equations were generated using Excel 2007.

2.1 Effects of Dietary Vitamin C on Growth Performance, Morphological Indices, and Feed Utilization

As shown in Table 2 , weight gain rate and specific growth rate of juvenile striped bass in the 74.03 mg/kg group were significantly higher than those in the 10.97, 618.88, and 910.98 mg/kg groups ($P<0.05$), with no significant differences from other groups ($P>0.05$). Both weight gain rate and specific growth rate decreased as dietary vitamin C increased from 74.03 to 910.98 mg/kg, with growth inhibition observed at 910.98 mg/kg. Survival rate was not significantly affected by dietary vitamin C content ($P>0.05$).

Feed conversion ratio in the 74.03 mg/kg group was significantly lower than in the 10.97 and 910.98 mg/kg groups ($P<0.05$), while the 618.88 mg/kg group had a significantly lower FCR than the 910.98 mg/kg group ($P<0.05$). No significant differences were observed among remaining groups ($P>0.05$). Protein efficiency ratio in the 10.97 and 910.98 mg/kg groups was significantly lower than in the 74.03 and 618.88 mg/kg groups ($P<0.05$). Condition factor in the 335.33 mg/kg group was significantly lower than in the 74.03, 148.33, and 213.64 mg/kg groups ($P<0.05$), but did not differ significantly from the 10.97, 618.88, and 910.98 mg/kg groups ($P>0.05$). Hepatosomatic index was not significantly affected by dietary vitamin C ($P>0.05$), while viscerosomatic index in the 74.03 mg/kg group was significantly higher than in the 910.98 mg/kg group ($P<0.05$).

Broken-line regression analysis between dietary vitamin C content and specific growth rate (Figure 1 [Figure 1: see original paper]) yielded two equations: $Y = 0.0054X + 0.7609$ ($R^2 = 1.0000$) and $Y = -0.0005X + 1.1542$ ($R^2 = 0.9478$). The intersection point indicated the optimal dietary vitamin C level for maximum specific growth rate, which was 66.66 mg/kg.

2.2 Effects of Dietary Vitamin C on Serum Biochemical Indices

As shown in Table 3 , serum total protein content in the 10.97 and 74.03 mg/kg groups was significantly lower than in other groups ($P<0.05$), with no significant differences among the remaining groups ($P>0.05$). Serum triglyceride content in the 618.88 and 910.98 mg/kg groups was significantly lower than in other groups ($P<0.05$). Serum lysozyme activity in the 10.97 mg/kg group was significantly lower than in the 213.64, 335.33, 618.88, and 910.98 mg/kg groups ($P<0.05$). Dietary vitamin C content had no significant effect on serum total cholesterol or alkaline phosphatase activity ($P>0.05$).

Broken-line regression analysis between dietary vitamin C content and serum lysozyme activity (Figure 2 [Figure 2: see original paper]) produced the equations $Y = 0.2365X + 43.694$ ($R^2 = 0.8938$) and $Y = -0.0547X + 144.19$ ($R^2 =$

1.0000). The intersection point indicated the dietary vitamin C requirement for maximum serum lysozyme activity was 345.11 mg/kg.

2.3 Effects of Dietary Vitamin C on Serum Antioxidant Indices

As shown in Table 4 , serum SOD activity and T-AOC increased initially then decreased with rising dietary vitamin C, while serum MDA content showed the opposite trend. The 335.33 mg/kg group exhibited the highest serum SOD activity, significantly greater than the 10.97, 74.03, and 910.98 mg/kg groups ($P<0.05$). Serum T-AOC was highest in the 335.33 mg/kg group, significantly higher than in the 10.97, 618.88, and 910.98 mg/kg groups ($P<0.05$). Serum MDA content was lowest in the 335.33 mg/kg group, significantly lower than in the 10.97 mg/kg group ($P<0.05$). Dietary vitamin C content did not significantly affect serum CAT activity ($P>0.05$).

These results indicate that a dietary vitamin C level of 335.33 mg/kg was most effective for improving serum antioxidant indices in juvenile striped bass.

2.4 Effects of Dietary Vitamin C on Liver Antioxidant Indices

As shown in Table 5 , the 335.33 mg/kg group exhibited the highest liver SOD and CAT activities and the lowest MDA content. Dietary vitamin C content did not significantly affect liver T-AOC ($P>0.05$). Liver SOD and CAT activities in the 10.97 mg/kg group were significantly lower than in all other groups ($P<0.05$). Liver MDA content in the 148.33, 335.33, and 910.98 mg/kg groups was significantly lower than in the 10.97 mg/kg group ($P<0.05$).

These findings demonstrate that a dietary vitamin C level of 335.33 mg/kg significantly enhanced the antioxidant capacity of liver tissue in juvenile striped bass.

2.5 Effects of Dietary Vitamin C on Muscle Antioxidant Indices

As shown in Table 6 , dietary vitamin C content did not significantly affect muscle CAT activity or MDA content ($P>0.05$). However, muscle SOD activity and T-AOC in the 74.03 mg/kg group were significantly higher than in all other groups ($P<0.05$).

These results suggest that a dietary vitamin C level of 74.03 mg/kg effectively enhanced muscle antioxidant capacity.

2.6 Effects of Dietary Vitamin C on GOT and GPT Activities in Serum and Liver

As shown in Table 7 , serum GOT activity in the 213.64, 335.33, 618.88, and 910.98 mg/kg groups was significantly lower than in the 10.97 mg/kg group ($P<0.05$). Liver GOT activity increased initially then decreased with rising

dietary vitamin C, with the 10.97 mg/kg group showing significantly lower activity than all other groups ($P<0.05$) and peak activity observed in the 335.33 mg/kg group. Serum GPT activity in the 10.97 and 74.03 mg/kg groups was significantly higher than in other groups ($P<0.05$), with no significant differences among remaining groups ($P>0.05$). Dietary vitamin C content did not significantly affect liver GPT activity ($P>0.05$).

2.7 Effects of Dietary Vitamin C on Vitamin C Accumulation in Serum, Liver, and Muscle

As shown in Table 8 , serum vitamin C accumulation increased progressively with dietary vitamin C, reaching maximum levels in the 910.98 mg/kg group, which was significantly higher than all other groups ($P<0.05$). Liver vitamin C accumulation also increased with dietary vitamin C, with the 910.98 mg/kg group showing significantly higher levels than the 10.97, 74.03, 148.33, and 213.64 mg/kg groups ($P<0.05$), but not differing significantly from the 335.33 and 618.88 mg/kg groups ($P>0.05$). Muscle vitamin C accumulation increased with dietary vitamin C, peaking in the 618.88 mg/kg group, which was significantly higher than the 10.97, 74.03, 148.33, and 213.64 mg/kg groups ($P<0.05$), but not significantly different from the 335.33 and 910.98 mg/kg groups ($P>0.05$).

Liver vitamin C accumulation was the highest among the three tissues examined. Broken-line regression analysis between dietary vitamin C content and liver vitamin C accumulation (Figure 3 [Figure 3: see original paper]) yielded the equations $Y = 0.0585X + 7.5367$ ($R^2 = 0.9967$) and $Y = -0.0017X + 25.141$ ($R^2 = 1.0000$). The intersection point indicated the dietary vitamin C requirement for maximum liver vitamin C accumulation was 309.93 mg/kg.

2.8 Effects of Dietary Vitamin C on Muscle Composition

As shown in Table 9 , dietary vitamin C content did not significantly affect muscle crude protein, crude lipid, or ash content ($P>0.05$). Muscle moisture content in the 74.03 mg/kg group was significantly lower than in the 10.97, 148.33, and 213.64 mg/kg groups ($P<0.05$). Muscle collagen content increased with dietary vitamin C, with the 910.98 mg/kg group showing significantly higher levels than all other groups ($P<0.05$).

3.1 Effects of Dietary Vitamin C on Growth Performance and Serum Biochemical Indices

The results demonstrate that appropriate dietary vitamin C supplementation significantly promoted growth in juvenile striped bass, while excessive levels (910.98 mg/kg) inhibited growth. This finding aligns with studies on orange-spotted grouper (*Epinephelus coioides*) [13] and hybrid tilapia (*Oreochromis*

niloticus × *O. aureus*) [14]. Broken-line regression analysis indicated an optimal dietary vitamin C requirement of 66.66 mg/kg based on specific growth rate, which is consistent with the 63 mg/kg requirement reported for black carp (*Mylopharyngodon piceus*) [15]. During the trial, control group fish did not exhibit typical vitamin C deficiency symptoms such as fin erosion, spinal curvature, internal hemorrhage, or increased mortality. This may be attributed to the basal diet containing some vitamin C (10.97 mg/kg) or to the relatively large fish size being less sensitive to deficiency. Additionally, Lee et al. [16] reported that vitamin C deficiency symptoms in Korean rockfish (*Sebastes schlegeli) only appeared after 12–16 weeks of feeding a vitamin C-free diet, suggesting that longer experimental periods may be needed to confirm deficiency signs in striped bass. Although growth performance differed among treatments, overall growth was modest with relatively low weight gain rates, likely due to the large initial fish size. Future studies should use smaller fish to achieve more pronounced effects.

Alkaline phosphatase (AKP) is a key metabolic regulatory enzyme that participates in phosphate transport and calcium-phosphorus metabolism, playing a crucial role in nutrient absorption and utilization. It also enhances pathogen recognition and phagocytosis by modifying pathogen surface structures, thereby strengthening immunity [17]. The current study found no significant effect of dietary vitamin C on serum AKP activity, possibly because fish primarily mobilize tissue antioxidant systems in response to external stress, while serum AKP is mainly involved in metabolic regulation and nutrient utilization rather than immune responses. The underlying mechanisms require further investigation.

3.2 Effects of Dietary Vitamin C on Tissue Antioxidant Indices and Serum/Liver GOT and GPT Activities

The formation and elimination of reactive oxygen species must maintain dynamic equilibrium to protect organisms from oxidative damage and ensure normal physiological function. Malondialdehyde (MDA) is a key product of lipid peroxidation that exhibits strong cytotoxicity by damaging cellular structure and function [18]. MDA content directly reflects the level of oxidative damage to biological membranes and indirectly indicates the degree of free radical damage [19]. The antioxidant enzyme system, including SOD and CAT, scavenges excess free radicals generated during metabolism. SOD specifically catalyzes the dismutation of superoxide anions (O) into hydrogen peroxide and oxygen, while CAT converts hydrogen peroxide into non-toxic water and oxygen. Therefore, measuring SOD, CAT activities, and MDA content accurately reflects the antioxidant status in fish [20]. Total antioxidant capacity (T-AOC) evaluates the ability to scavenge free radicals and other potentially toxic oxidants, with higher values indicating stronger defense capacity.

In muscle tissue of juvenile striped bass, the 335.33 mg/kg group showed maximum SOD activity. Although dietary vitamin C did not significantly affect muscle CAT activity or MDA content, the 74.03 mg/kg group exhibited the strongest T-AOC. This may be because other antioxidant factors such as glutathione per-

oxidase (GSH-Px) also contributed to the antioxidant response. Additionally, vitamin C did not significantly affect CAT activity in serum or muscle, possibly because SOD is the primary enzyme acting on reactive oxygen radicals, while CAT is a downstream enzyme that scavenges hydrogen peroxide produced by SOD. Since GSH-Px can also perform this function, CAT may not have been mobilized in serum and muscle tissues.

Previous studies reported that dietary vitamin C at 316, 2,000, 100, and 630 mg/kg enhanced SOD activity in tissues of yellow catfish (*Pelteobagrus fulvidraco*) [21], largemouth bass (*Micropterus salmoides*) [22], grass carp (*Ctenopharyngodon idellus*) [23], and orange-spotted grouper [24]. However, Song et al. [25] found no significant effect of dietary vitamin C on SOD activity in Chinese shrimp (*Fenneropenaeus chinensis*), while Ai [26] reported decreased serum SOD activity with increasing dietary vitamin C in Chinese mitten crab (*Eriocheir sinensis*). These inconsistent results may be related to species differences, developmental stage, health status, culture environment, vitamin C source, and interactions with other nutrients such as vitamin E. The specific mechanisms require further investigation.

Glutamic oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) are primarily distributed in liver and cardiac tissues under normal conditions, with low serum activity. They participate in intracellular protein metabolism and transamination. When liver damage occurs, increased cell membrane permeability releases GOT and GPT into the bloodstream, elevating serum transaminase activities, which thus reflect hepatic pathology [27]. In this study, serum GPT activity decreased with increasing dietary vitamin C before stabilizing. Liver GOT activity increased initially then decreased at 910.98 mg/kg. These results suggest that appropriate dietary vitamin C supplementation provides hepatoprotective effects.

3.3 Effects of Dietary Vitamin C on Tissue Vitamin C Accumulation and Muscle Nutritional Composition

Wang et al. [28] reported that tissue vitamin C accumulation in fish follows the pattern: muscle < liver < brain < gills. Our results are consistent, with muscle showing the lowest accumulation among the three tissues examined. Al-Amoudi et al. [29] attributed this pattern to muscle activity, as vitamin C exists in a consumable form that directly participates in physiological processes. The liver serves as the primary site for vitamin C metabolism and storage, resulting in much higher concentrations than muscle. Dabrowski [30] proposed that dietary vitamin C is primarily used to maintain stable tissue concentrations, suggesting that the level required to achieve maximum tissue saturation represents the optimal requirement. Fournier et al. [31] reported that hepatic ascorbic acid saturation is the most reliable criterion for estimating vitamin C requirements in European sea bass (*Dicentrarchus labrax*). Therefore, the 335.33 mg/kg level in this study represents a credible indicator. Broken-line regression analysis indicated that liver vitamin C accumulation reached a plateau at 309.93 mg/kg

dietary vitamin C.

Dietary vitamin C did not significantly affect muscle crude protein, crude lipid, or ash content, consistent with findings in half-smooth tongue sole (*Cynoglossus semilaevis*) [32], topmouth culter (*Culter alburnus*) [33], and GIFT tilapia (*O. niloticus*) [34]. However, studies on orange-spotted grouper [9] reported significant effects on muscle crude lipid. High dietary vitamin C significantly increased muscle collagen content. Collagen is rich in hydroxyproline and hydroxylysine, and vitamin C serves as an essential cofactor for the hydroxylation of proline and lysine, promoting collagen synthesis.

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4 Conclusion

1. Appropriate dietary vitamin C supplementation significantly affected growth performance, feed utilization, serum biochemistry, tissue antioxidant indices, and muscle collagen content in juvenile striped bass.
2. Based on specific growth rate, liver vitamin C accumulation, and serum lysozyme activity as evaluation criteria, broken-line regression analysis indicated that the dietary vitamin C requirements for juvenile striped bass (approximately 88 g) were 66.66, 309.93, and 345.11 mg/kg, respectively.

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Note: Figure translations are in progress. See original paper for figures.