

Effects of Dietary Copper Supplementation on Growth and Body Color of Channel Catfish (*Ictalurus punctatus*) in Practical Diets (Postprint)

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Abstract

Five experimental diets were prepared by supplementing a practical diet (containing 11.1 mg/kg copper) with 0 (control), 5, 10, 20, and 40 mg/kg copper [as copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$)], and fed to channel catfish with an average body weight of (98.1 ± 0.5) g for 42 days to investigate the effects of dietary copper supplementation on growth and body color of channel catfish. Each diet was assigned to three replicates, with 20 fish per replicate. The results showed that compared with the control group, dietary supplementation of 10 mg/kg copper significantly increased the weight gain rate ($P < 0.05$) and significantly decreased the feed conversion ratio ($P < 0.05$) of fish; when dietary copper supplementation was further increased to 40 mg/kg, the weight gain rate significantly decreased compared with the 10 mg/kg supplementation group ($P < 0.05$), and the feed conversion ratio significantly increased ($P < 0.05$). Liver and bone copper contents increased with increasing dietary copper supplementation, with liver copper content in the 20 and 40 mg/kg copper supplementation groups being significantly higher than that in the control and 5 mg/kg copper supplementation groups ($P < 0.05$), and bone copper content in the 40 mg/kg copper supplementation group also being significantly higher than that in the control group ($P < 0.05$), while muscle copper content remained essentially unchanged ($P > 0.05$). Dietary copper supplementation of 0–40 mg/kg had no significant effects on dorsal skin, muscle color values, total lutein content, or dorsal skin tyrosinase activity of channel catfish ($P > 0.05$). There were no significant differences among groups in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, total bilirubin (T-Bil) content, or muscle moisture, crude protein, crude lipid, and crude ash contents ($P > 0.05$). The 10 mg/kg copper supplementation group exhibited the highest serum copper-zinc superoxide dismutase (Cu,Zn-SOD) activity, which was significantly higher than that in all other groups ($P < 0.05$), while no significant differences were observed

among the other groups ($P>0.05$). In summary, under the experimental conditions of this study, the recommended copper supplementation level in practical diets for channel catfish is 10 mg/kg (total dietary copper content 20.2 mg/kg).

Full Text

Effects of Copper Supplementation in Practical Diets on Growth and Body Color of Channel Catfish

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Abstract: A practical diet containing 11.1 mg/kg copper was supplemented with 0 (control), 5, 10, 20, or 40 mg/kg copper (as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) to formulate five experimental diets, which were fed to channel catfish (*Ictalurus punctatus*) with an average body weight of (98.1 ± 0.5) g for 42 days to investigate the effects of dietary copper supplementation on growth and body color. Each diet was assigned to three replicate groups, with 20 fish per replicate. The results showed that compared with the control group, dietary supplementation of 10 mg/kg copper significantly increased weight gain rate ($P<0.05$) and significantly decreased feed conversion ratio ($P<0.05$). However, when copper supplementation increased to 40 mg/kg, weight gain rate was significantly reduced ($P<0.05$) and feed conversion ratio was significantly elevated ($P<0.05$) compared with the 10 mg/kg group.

Liver and bone copper contents increased with increasing dietary copper levels. Specifically, liver copper content in the 20 and 40 mg/kg groups was significantly higher than in the control and 5 mg/kg groups ($P<0.05$), and bone copper content in the 40 mg/kg group was significantly higher than in the control group ($P<0.05$), while muscle copper content remained essentially unchanged ($P>0.05$). Dietary copper supplementation from 0 to 40 mg/kg had no significant effects on dorsal skin or muscle color values, total xanthophylls content, or dorsal skin tyrosinase activity in channel catfish ($P>0.05$). No significant differences were observed among groups in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, total bilirubin (T-Bil) content, or muscle moisture, crude protein, crude lipid, and ash contents ($P>0.05$).

The 10 mg/kg copper supplementation group exhibited the highest serum

copper-zinc superoxide dismutase (Cu,Zn-SOD) activity, which was significantly higher than all other groups ($P < 0.05$), while no significant differences were found among the remaining groups ($P > 0.05$). In conclusion, under the present experimental conditions, the recommended copper supplementation level in practical diets for channel catfish is 10 mg/kg, yielding a total dietary copper content of 20.2 mg/kg.

Keywords: channel catfish; copper; growth; body color

Copper is an essential trace element for fish that plays vital roles in various life activities. As a cofactor for enzymes such as cytochrome C oxidase (CCO), monoamine oxidase (MAO), and copper-zinc superoxide dismutase (Cu,Zn-SOD), copper participates in physiological processes including hematopoiesis, free radical defense, connective tissue biosynthesis, and cellular respiration [1]. The NRC (2011) [2] recommends dietary copper requirements of 3 mg/kg for common carp, 5 mg/kg for channel catfish, and 5–10 mg/kg for Atlantic salmon. Copper deficiency in diets can lead to reduced growth in common carp [3] and grouper [4], decreased activities of cytochrome C oxidase and superoxide dismutase in the heart of channel catfish [5], and notably reduced liver copper content in Atlantic salmon suffering from cold-water disease (“Hitra disease”) [6]. However, excessive copper intake can also reduce fish growth rate and feed utilization efficiency while increasing copper accumulation in the liver [5,7-9]. High concentrations of copper in water can cause hemorrhage at body surfaces such as fin bases, tails, and gills, as well as hepatopancreatic necrosis [10], though such toxic effects have not been reported from dietary copper excess, possibly due to the barrier function of intestinal mucosa against toxic metals [11].

Channel catfish, also known as channel cat, belongs to the family Ictaluridae in the order Siluriformes and is native to North America. Characterized by rapid growth, broad feeding habits, and delicious meat without intermuscular bones—making it suitable for fillet processing—channel catfish has become an important freshwater aquaculture species in China. In recent years, abnormal body coloration has frequently occurred in cultured channel catfish, with multifactorial causes. Given that copper ions serve as a cofactor for tyrosinase, the key enzyme regulating melanin production, we hypothesized whether this color abnormality might be related to copper deficiency or excess. Therefore, this study investigated the effects of different dietary copper supplementation levels on growth and body color of channel catfish fed practical diets.

1.1 Experimental Design and Diets

A practical basal diet was formulated using fish meal, soybean meal, cottonseed meal, rapeseed meal, wheat middling, wheat bran, and other ingredients to contain 31% crude protein. Copper (as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) was supplemented at 0 (control), 5, 10, 20, or 40 mg/kg to produce five experimental diets with

measured copper concentrations of 11.1, 16.0, 20.2, 28.9, and 44.2 mg/kg, respectively. Feed ingredients were ground through a 40-mesh sieve, thoroughly mixed, and processed into 2-mm floating extruded pellets using an extruder (SLP-45, developed by the Fishery Machinery and Instrument Research Institute, Chinese Academy of Fishery Sciences) at a temperature of $(110 \pm 5)^\circ\text{C}$. The pellets were air-dried and stored at 4°C until use. The composition and nutrient levels of the basal diet are presented in Table 1 .

1.2 Experimental Fish and Culture Management

The feeding trial was conducted at the Special Aquaculture Base of Shanghai Ocean University. Experimental fish were purchased from Chaohu Aquaculture Farm in Anhui Province and acclimated to the basal diet for one week prior to the trial. Three hundred healthy channel catfish with an average body weight of (98.1 ± 0.5) g were randomly stocked into 15 net cages ($2.5 \text{ m} \times 1.2 \text{ m} \times 1.0 \text{ m}$, mesh size $5 \text{ mm} \times 5 \text{ mm}$) positioned in the same indoor cement tank, with three replicates per dietary treatment and 20 fish per replicate. Fish were hand-fed three times daily (08:00, 12:00, and 17:00) at a feeding rate of 3-5% body weight, which was adjusted appropriately based on growth and feeding behavior to maintain consistent feeding levels across all cages. During the 6-week trial, one-third of the water was exchanged every two days using filtered pond water. Water temperature was maintained at $24\text{-}28^\circ\text{C}$, pH at 7-8, dissolved oxygen concentration above 6.0 mg/L, and ammonia nitrogen concentration below 0.2 mg/L.

Table 1 Composition and nutrient levels of the basal diet (air-dry basis)

Item	Content
Ingredients	
Fish meal	
Soybean meal	
Cottonseed meal	
Rapeseed meal	
Wheat bran	
Rice bran	
Wheat middling	
Soybean oil	
$\text{Ca}(\text{H}_2\text{PO}_4)_2$	
L-ascorbate-2-monophosphate (35%)	
Inositol	
Vitamin premix ²	
Mineral premix ³	
Choline chloride (50%)	
Total	
Nutrient levels	
Dry matter (DM)	

Item	Content
Crude protein (CP)	
Ether extract (EE)	
Ash	

¹The ingredients were purchased from Shanghai Nonghao Feed Co., Ltd. The crude protein content of fish meal (Peru), soybean meal, rapeseed meal, and cottonseed meal was 67.6%, 46.5%, 35.6%, and 45.0%, respectively.

²The vitamin premix provided the following per kg of diet: VA 6,000 IU, VD 2,000 IU, VE 50 IU, VK 5 mg, VB₁ 15 mg, VB₂ 15 mg, VB₃ 30 mg, VB₅ 35 mg, VB₆ 6 mg, biotin 0.2 mg, folic acid 3 mg, VB₁₂ 0.03 mg.

³The mineral premix provided the following per kg of diet: Ca(IO₃)₂ 0.04 g, CoCl₂ · 6H₂O 0.01 g, FeSO₄ · H₂O 0.446 g, ZnSO₄ · H₂O 0.232 g, MnSO₄ · H₂O 0.063 g, Na₂SeO₃ · 5H₂O 0.01 g, MgSO₄ · 7H₂O 0.645 g.

1.3.1 Growth and Morphological Indices Calculation

At the end of the trial, fish were fasted for 24 h. The number and total weight of fish in each cage were recorded to calculate weight gain rate (WGR), feed conversion ratio (FCR), and survival rate (SR). Three fish were randomly selected from each cage to measure body length and weight. After dissection, viscera and liver weights were measured to calculate hepatosomatic index (HSI), viscerosomatic index (VSI), and condition factor (CF).

Weight gain rate (%) = $100 \times [\text{final body weight (g)} - \text{initial body weight (g)}] / \text{initial body weight (g)}$

Feed conversion ratio = $\text{total feed intake (g)} / [\text{final body weight (g)} - \text{initial body weight (g)}]$

Survival rate (%) = $100 \times \text{final fish number} / \text{initial fish number}$

Hepatosomatic index (%) = $100 \times \text{liver weight (g)} / \text{body weight (g)}$

Viscerosomatic index (%) = $100 \times \text{viscera weight (g)} / \text{body weight (g)}$

Condition factor (g/cm³) = $100 \times \text{body weight (g)} / \text{body length (cm)}^3$

1.3.2 Proximate Composition Analysis of Feed and Muscle

At the end of the trial, three fish were randomly collected from each cage. Dorsal muscle above the lateral line below the dorsal fin was sampled and stored at -20°C for proximate composition analysis. Moisture content in feed and muscle samples was determined by oven drying at 105°C (GB/T 5009.3–2003). Crude protein content was measured using the Kjeldahl method (GB/T 5009.5–2003). Crude lipid content was determined by chloroform-methanol extraction according to Folch et al. [12]. Ash content was measured using a muffle furnace (GB/T 5009.4–2003).

1.3.3 Color Value Determination of Skin and Muscle

At the end of the trial, three fish were randomly selected from each cage. After surface moisture was removed with absorbent paper, a colorimeter probe (WSC-S, Shanghai Precision Scientific Instrument Co., Ltd.) was placed directly on the dorsal skin above the lateral line to measure skin color values. The skin was then removed, and the probe was placed on the dorsal muscle above the lateral line to measure muscle color values, recording lightness (L), redness (a), and yellowness (b*) values.

1.3.4 Determination of Total Xanthophylls Content in Skin and Muscle

After color measurement, 2-3 g of skin and muscle samples were collected for total xanthophylls content determination using the method of Quackenbush et al. [13]. Briefly, samples were minced and placed in a 25-mL brown volumetric flask with 7.5 mL extraction solution (n-hexane:acetone:anhydrous ethanol:toluene = 10:7:6:7). The flask was sealed and shaken for 1 min, then 1 mL of 40% KOH-methanol solution was added and shaken for another 1 min. The mixture was heated in a 55.5°C water bath for 20 min (with cooling of the flask neck to prevent solvent loss), cooled, and stored in darkness for 1 h. After adding 7.5 mL n-hexane and shaking for 1 min, the solution was diluted to 25 mL with 10% sodium sulfate solution, vigorously shaken for 1 min, and stored in darkness for 1 h. The absorbance of the supernatant was measured at 474 nm using a spectrophotometer, and total xanthophylls content was calculated based on a standard curve.

1.3.5 Tyrosinase Activity Assay in Dorsal Skin

Approximately 1 g of dorsal skin was homogenized (ice water bath) with 67 mmol/L phosphate buffer (pH 6.8) at a 1:5 ratio. The homogenate was centrifuged at 8,000 r/min for 25 min at 4°C, and the supernatant was collected for tyrosinase activity determination according to Ding Yuting et al. [14]. The assay procedure was as follows: 0.5 mL of 3 mg/mL L-dopa was added to 2 mL pre-warmed (28°C) supernatant, bringing the total reaction volume to 2.5 mL. The absorbance at 475 nm was measured immediately after mixing and again after 10 min at room temperature.

Tyrosinase activity was calculated using the following formula:

$$\text{Enzyme activity (U)} = (\Delta\text{OD}_{475} \times V) / (0.001 \times T)$$

Where: ΔOD_{475} is the difference between the two absorbance measurements ($\Delta\text{OD}_{475} = \Delta\text{OD}_{10} - \Delta\text{OD}_0$); V is the sample volume; T is the time interval between measurements.

1.3.6 Serum Biochemical Indices Determination

At the end of the trial, three fish were randomly selected from each cage. Blood was collected from the caudal vein and centrifuged at 3,000 r/min for 15 min.

Serum was collected and stored at -80°C . Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin (T-Bil) contents were measured using a Mindray BS-200 automatic biochemical analyzer. Serum Cu,Zn-SOD activity was determined using assay kits from Nanjing Jiancheng Bioengineering Institute.

1.3.7 Tissue Copper Content Determination

Tissue copper content was determined by atomic absorption spectrometry according to Zhang Yunhua [15]. Briefly, 1 g of sample was accurately weighed and placed in a crucible, carbonized on an electric furnace until no smoke was produced, then ashed in a muffle furnace at 550°C for 6 h. After cooling to room temperature, 10 mL of mixed acid (nitric acid:perchloric acid = 4:1) was added and left for over 5 h. The mixture was carefully heated on an electric furnace until the ashed sample dissolved (black carbon particles disappeared) and the solution was nearly evaporated to dryness. The crystals were dissolved in 1% HCl solution, transferred to a 25-mL volumetric flask, and diluted to volume for measurement. A blank control was prepared using the same procedure without sample. Copper content was measured using a TAS-900 atomic absorption spectrophotometer (Beijing Puxi General Instrument Co., Ltd.).

1.4 Statistical Analysis

Experimental data were processed and analyzed using SPSS 17.0 statistical software. Results are expressed as mean \pm standard deviation (SD). One-way ANOVA was performed, followed by Duncan's multiple comparison test. Differences were considered significant at $P < 0.05$.

2.1 Growth and Morphological Indices

After 6 weeks of culture, the growth and morphological indices of channel catfish in each group are shown in Table 2. Weight gain rate exhibited a trend of initial increase followed by decrease with increasing copper supplementation. Specifically, the 10 mg/kg copper group showed significantly higher weight gain rate and significantly lower feed conversion ratio compared with the control group ($P < 0.05$). The 40 mg/kg copper group exhibited significantly lower final body weight and weight gain rate compared with the 10 mg/kg group ($P < 0.05$), and significantly higher feed conversion ratio compared with the control group ($P < 0.05$). No significant differences were observed in survival rate among all groups ($P > 0.05$), with all groups achieving 100% survival. No significant differences were found in morphological indices among groups ($P > 0.05$).

Table 2 Effects of dietary copper supplemental level on growth and morphological indices of channel catfish

Item	Copper supplemental level (mg/kg)
Initial body weight (IBW, g)	98.0 \pm 0.8 98.1 \pm 0.3 98.6 \pm 1.2 98.1 \pm 0.4 97.8 \pm 0.6 <i>Finalbodyweight(FBW, g)</i> 217.7 \pm 4.7 ^a 218.5 \pm ...

Values in the same row with no letter or the same letter superscripts indicate no significant difference ($P>0.05$), while different lowercase letters indicate significant difference ($P<0.05$). The same applies below.

2.2 Proximate Composition of Muscle

As shown in Table 3, no significant differences were observed among groups in muscle moisture, crude protein, crude lipid, or ash contents ($P>0.05$).

Table 3 Effects of dietary copper supplemental level on muscle proximate composition of channel catfish (fresh weight basis)

Item	Copper supplemental level (mg/kg)
Moisture	76.74 \pm 0.91 77.10 \pm 0.67 76.68 \pm 0.81 76.27 \pm 0.95 76.84 \pm 0.93 <i>Crudeprotein(CP)</i> 18.62 \pm 0.34 18.9...

2.3 Color Values, Total Xanthophylls Content, and Tyrosinase Activity in Dorsal Skin and Muscle

As shown in Table 4, no significant differences were observed among groups in color values, total xanthophylls content of dorsal skin and muscle, or tyrosinase activity in dorsal skin ($P>0.05$).

Table 4 Effects of dietary copper supplemental level on color values and total xanthophylls content of dorsal skin and muscle, and tyrosinase activity of dorsal skin

Item	Copper supplemental level (mg/kg)
Dorsal skin	
L*	20.48 \pm 2.6 19.35 \pm 1.9 21.38 \pm 2.2 21.28 \pm 1.9 21.43 \pm 1.6 <i>a</i> * 3.53 \pm 1.20 3.10 \pm 1.31 2.67 \pm 0.71 2.50 \pm 0.83 2.11 \pm 0.63 <i>b</i> * 2.48 \pm 0.73 2.02 \pm 1.09 2.72 \pm 1.21 2.44 \pm 0.5 2.02 \pm 0.91 <i>Totalxanthophylls(mg/kg)</i> 2.75 \pm 0.05 2.81... * <i>Dorsalmuscle</i> * * <i>L</i> * 50.76 \pm 2.6 50.72 \pm 2.8 51.68 \pm 2.5 51.61 \pm 2.7 49.37 \pm 2.4 <i>a</i> * 3.59 \pm 0.89 4.54 \pm 0.96 4.22 \pm 0.93 3.96 \pm 1.01 3.42 \pm 1.13 <i>b</i> * 3.50 \pm 0.99 4.08 \pm 1.48 4.49 \pm 0.68 3.91 \pm 1.16 4.13 \pm 0.98 <i>Totalxanthophylls(mg/kg)</i> 1.06 \pm 0.26 1.18...

2.4 Serum Biochemical Indices

As shown in Table 5, no significant differences were observed among groups in serum AST and ALT activities or T-Bil content ($P > 0.05$). The 10 mg/kg copper supplementation group exhibited the highest serum Cu,Zn-SOD activity, which differed significantly from all other groups ($P < 0.05$), while no significant differences were found among the remaining groups ($P > 0.05$).

Table 5 Effects of dietary copper supplemental level on serum biochemical indices of channel catfish

Item	Copper supplemental level (mg/kg)
Aspartate amino-transferase (AST, U/L)	24.85 \pm 3.56 24.25 \pm 2.85 25.60 \pm 3.26 24.72 \pm 3.97 24.06 \pm 1.62 <i>Alanineaminotransferase(ALT, U/L)</i>
	<i>Bil, μmol/L</i> 2.54 \pm 0.42 2.43 \pm 0.53 2.29 \pm 0.36 2.20 \pm 0.64 2.43 \pm 0.51 <i>Cu, Zn-SOD(U/mL)</i> 58.02 \pm 1.83 56.00 \pm 2.14 64.01 \pm 3.72 55.56 \pm 2.97 56.37 \pm 2.71

2.5 Tissue Copper Content

As shown in Table 6, no significant differences were observed among groups in muscle copper content ($P > 0.05$). Liver and bone copper contents increased with increasing dietary copper supplementation. Specifically, liver copper content in the 20 and 40 mg/kg groups was significantly higher than in the control and 5 mg/kg groups ($P < 0.05$), and bone copper content in the 40 mg/kg group was significantly higher than in the control group ($P < 0.05$).

Table 6 Effects of dietary copper supplemental level on tissue copper content of channel catfish (mg/kg)

Item	Copper supplemental level (mg/kg)
Muscle copper	0.80 \pm 0.06 0.74 \pm 0.06 0.71 \pm 0.07 0.86 \pm 0.01 0.83 \pm 0.11 <i>Livercopper</i> 3.46 \pm 0.04 ^a 3.46 \pm 0.15 ^a 3.56 \pm 0.05 ^a

3.1 Effects of Dietary Copper Supplementation on Growth and Morphological Indices of Channel Catfish

Gatlin et al. [16] investigated the copper requirement of channel catfish (83 \pm 3 g) using purified diets and found that heart cytochrome C oxidase and superoxide dismutase activities were significantly higher when dietary copper exceeded 4 mg/kg compared with 0 or 2 mg/kg, though growth indices showed no significant differences, leading to a recommended minimum dietary copper requirement of 5 mg/kg—the standard adopted by NRC (2011). In the present study, channel catfish fed the unsupplemented practical diet exhibited relatively slow growth,

while supplementation of 10 mg/kg copper significantly improved growth performance. However, when copper supplementation reached 40 mg/kg, growth performance declined. Additionally, serum Cu,Zn-SOD activity peaked at 10 mg/kg copper supplementation. Cu,Zn-SOD is an antioxidant enzyme that protects cells from free radical damage [17]. Wang et al. [18] reported that in abalone (*Haliotis discus hannai*) fed diets containing 1.08, 3.76, 6.54, 14.80, 26.84, and 109.41 mg/kg copper for 24 weeks, liver and serum Cu,Zn-SOD activities increased initially then decreased with increasing dietary copper. Similar patterns of liver Cu,Zn-SOD activity were observed in channel catfish [16] and grouper [20]. Lin et al. [4] suggested that free copper in the body can damage superoxide dismutase, which may explain why high dietary copper reduces serum Cu,Zn-SOD activity in fish.

Based on growth performance, the optimal dietary copper supplementation level in this study was 10 mg/kg, yielding a total dietary copper content of 20.2 mg/kg—substantially higher than the NRC (2011) recommendation. This discrepancy may be attributed to the fact that Gatlin et al. [16] used a purified diet containing 0.89 mg/kg copper, whereas the present study used a practical diet containing 11.1 mg/kg copper, mostly in bound forms with low bioavailability that could not meet growth requirements. Supplementation with 10 mg/kg inorganic copper improved growth performance, but antinutritional factors such as phytic acid in the practical diet likely reduced the utilization of supplemented inorganic copper, resulting in a higher copper requirement than the NRC (2011) recommendation.

Murai et al. [20] reported that channel catfish (14.5 ± 1.4 g) fed diets containing 16 and 32 mg/kg copper for 16 weeks fed 40 mg/kg copper for 13 weeks. This difference may be related to fish size, with smaller fish being less tolerant to high copper levels. Rainbow trout fed 700 mg/kg copper exhibited reduced growth primarily due to decreased feed intake [21], whereas high copper (35 mg/kg) in Atlantic salmon did not reduce feed intake but stimulated intestinal cells, impairing digestion and absorption and consequently reducing growth performance [22]. De Boeck et al. [5] suggested that high copper intake increased energy expenditure for maintenance at the expense of growth in common carp. In this study, 40 mg/kg copper supplementation reduced growth performance in channel catfish without causing typical copper toxicity symptoms or significantly affecting feed intake, suggesting this level may be insufficient to induce overt copper poisoning.

Baker et al. [23] reported that mullet fed 2,400 mg/kg copper for 10 weeks showed significantly reduced hepatosomatic index and condition factor, likely resulting from severe liver damage and atrophy. In Atlantic salmon fed 5–1,750 mg/kg copper for 3 months, condition factor decreased significantly only when copper exceeded 900 mg/kg [8], indicating that condition factor is not a sensitive indicator of dietary copper response. In the present study, copper supplementation ranged from 0 to 40 mg/kg, far below the levels in these reports, and no effects on morphological indices were observed.

3.2 Effects of Dietary Copper Supplementation on Body Color of Channel Catfish

Studies on catfish (*Clarias fuscus*) showed that increasing dietary copper from 3.5 to 9.5 mg/kg tended to increase carotenoid and xanthophyll content in dorsal skin [24]. However, the present study found no significant effects of dietary copper on color values or total xanthophylls content in dorsal skin and muscle of channel catfish. The discrepancy may be related to the different natural body colors of these species—channel catfish typically have gray-black skin and white muscle, while catfish exhibit yellow or yellow-brown coloration.

Melanin synthesis in organisms proceeds through a complex biochemical pathway with tyrosine as substrate under tyrosinase catalysis. Zhuge et al. [25] proposed that fish body color results from the combined expression of melanin and carotenoid pigments, with black intensity directly related to tyrosinase activity. Xu et al. [26] reported that tissue tyrosinase activity in Taihe silky fowl increased initially then decreased with increasing dietary copper. Few studies have examined the effects of high copper on melanin in fish. In this study, copper supplementation from 0 to 40 mg/kg did not significantly affect tyrosinase activity in dorsal skin of channel catfish.

3.3 Effects of Dietary Copper Supplementation on Serum Biochemical Indices of Channel Catfish

ALT, AST, and T-Bil are commonly used clinical indicators of liver function, with serum ALT and AST activities reflecting hepatocellular damage and T-Bil content reflecting biliary excretion, secretion, and detoxification functions [27]. Rockfish fed diets containing 50–500 mg/kg copper showed no significant changes in serum AST and ALT activities after 30 days, but both activities increased significantly after 60 days [28]. Common carp fed 250–1,000 mg/kg copper for 60 days also exhibited significantly increased serum AST and ALT activities [29]. In this study, dietary copper supplementation did not affect serum AST, ALT, or T-Bil in channel catfish, possibly due to the relatively low copper levels and short experimental duration.

3.4 Effects of Dietary Copper Supplementation on Tissue Copper Content of Channel Catfish

Lorentzen et al. [30] considered liver copper accumulation the most sensitive indicator of copper status. Atlantic salmon fed diets containing 5, 35, and 700 mg/kg copper for 4 weeks showed significantly elevated liver copper content [23]. In common carp, liver copper content increased with dietary copper (0–1,000 mg/kg), while muscle copper increased significantly only when dietary copper reached 500 mg/kg [29]; similar results were reported by De Boeck et al. [5]. Additionally, Qiao [31] found that copper deposition in bone increased with dietary copper in cobia. In this study, liver and bone copper contents increased

with dietary copper supplementation, while muscle copper remained relatively stable.

Conclusion

Under the present experimental conditions, dietary copper supplementation from 0 to 40 mg/kg did not significantly affect body color, flesh color, or muscle copper content in channel catfish, while liver and bone copper contents increased with copper supplementation. Supplementation of 10 mg/kg copper significantly improved weight gain rate, reduced feed conversion ratio, and yielded the highest serum Cu,Zn-SOD activity. Therefore, the recommended copper supplementation level in practical diets for channel catfish is 10 mg/kg, yielding a total dietary copper content of 20.2 mg/kg.

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