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Estimation of dietary copper requirements of Coho salmon *Oncorhynchus kisutch* (Walbaum, 1792), and effects on the growth performance, tissue Cu content, antioxidant capacity and hematological parameters

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Copper (Cu) is an essential trace mineral for the growth of most farmed fish species. Since natural water typically contains low Cu levels, exogenous Cu supplementation may be required in intensive aquaculture systems to meet the nutritional requirements of certain fish species. A 10-week feeding experiment was conducted to evaluate the Cu requirement on growth performance, tissue Cu content, hematological parameters and anti-oxidant responses in coho salmon *Oncorhynchus kisutch* (Walbaum, 1792). In this experiment, six experimental diets supplemented with graded Cu ($CuSO_4$ used as Cu source) contents (0.20, 2.10, 3.70, 5.80, 7.75, and 9.85 mg/kg) to feed the fish (180.22 ± 0.41 g). Total 180 fish were randomly distributed across 18 individuals tank (10 fish/cage, water volume 1,000-L) fed three times a day. The result showed that the mortality and morphological indices were completely unaffected by the increasing Cu supplementation in the diet ($P > 0.05$). Whereas, the non-supplemented diet (0.20 mg Cu/kg) had a poor growth performance of the fish ($P < 0.05$), including the lowest final body weight and specific growth rate, the highest feed conversion ratio. No significant differences ($P > 0.05$) were observed in the proximate composition of muscle across graded dietary copper levels. However, increasing dietary Cu level induced Cu accumulation ($P < 0.05$), but higher Cu level in the diet (> 5.8 mg/kg) did not further increase of muscle and liver in coho salmon. Compared with the 0.20 Cu mg/kg in diet, the supplemented diet enhanced the antioxidant capacity in liver and serum, and decrease the content of malondialdehyde in liver ($P < 0.05$). Diet with 0.20-5.80 mg/kg supplemental Cu significantly increased the serum alkaline phosphatase and lysozyme activities, decrease the serum alanine aminotransferase and aspartate aminotransferase activities ($P < 0.05$), while higher dietary Cu level (> 5.8 mg/kg) showed the opposite trend. The broken-line analysis based on specific growth rate, liver Cu accumulation, copper-zinc superoxide dismutase in liver and serum, the appropriate dietary Cu level for coho salmon were estimated to be 5.29–5.92 mg/kg.

Keywords *Oncorhynchus kisutch*, Copper requirement, Anti-oxidant, Serum biochemical parameters, Growth

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Copper (Cu) is exist in nature extensively, which is a basic trace element essential for the growth of all animals, including fish[1]. It plays a physiological role in the form of coenzyme and participates in physiological activities such as antioxidant, immune, hematopoietic, and skeletal development in animals[2–4].

Gills and digestive tract are the main ways for most fish to absorb Cu[5]. Unlike minerals such as calcium that can be absorbed directly from the aquatic environment (particularly in marine species), copper must be obtained primarily through dietary sources due to: (i) its low ambient concentrations (freshwater: 0.5–5 μg/L; seawater: 0.1–0.5 μg/L), and (ii) competitive inhibition by divalent cations (Ca^{2+}/Mg^{2+}) at uptake sites[6,7]. Fish Cu nutrition also has its own characteristics. Supplementation of minerals in diet is deemed to the main way for fish to take in minerals, which were used to maintain growth, improving fitness, and essential for a variety of physiological functions[8–10]. However, Cu is an essential trace metal and has the double effects of nutrition and toxicity[11]. Based on previous research, both the deficiency and excess of Cu can damage the health and physiological function of several fish[12,13]. Insufficient Cu supplement in diet would reduce appetite, slow growth, and anemia even skeletal anomalies and increased oxidative stress[14–17]. On the contrary, excessive Cu supplement in diet may cause toxicity syndrome, including damage tissue, lipid per-oxidation, change intestinal cell proliferation and regeneration, and induced pathophysiological alterations[18–21]. In addition, the appropriate Cu requirement of fish was determined by species[18,22], size, life stage and environmental factors. Hence, it is crucial to regulate and maintain the levels of Cu to ensure they remain at safe and optimal levels for the specific species of fish in question.

Oxidative stress is the damage mechanism of the organisms to the adverse environment, which induced reactive oxygen species (ROSs) are highly toxic to organisms[23]. Fish produce cellular antioxidant defense mechanisms to resist ROSs damage, which mainly relies on enzymatic and non-enzymatic defense systems[24–26]. In fish, the antioxidant defense system is primarily mediated by enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR), and glutathione S-transferase (GST), which collectively mitigate oxidative stress. Indeed, Cu is a co-factor in many key enzymes (i.e. copper-zinc (Cu-Zn) SOD, ceruloplasmin) required to prevent oxidative damage[9,14,27]. In recent years, research on the antioxidant system response of fish with dietary Cu levels have been explored[14,25,28–30]. In blunt snout bream *Megalobrama amblycephala*, yellowtail kingfish *Seriola lalandi* and in golden trout *Oncorhynchus mykiss aguabonita and* rainbow trout *Oncorhynchus mykiss*, inadequate and excessive Cu supplementation, both in feed or environment, can lead to reduced antioxidant enzyme activity[22,28,31,32]. Therefore, ensuring a healthy antioxidant status is essential for the safety of cultured coho salmon.

Coho salmon *Oncorhynchus kisutch* (Walbaum, 1792) is cold-water fish belonging to Salmoniformes, *Oncorhynchus*, which is famous for its rich protein, lipid, omega-3 fatty acids, and most beneficial for brain health also in humans[33,34]. With the rapid expansion of coho salmon aquaculture in China–evidenced by a 7-fold production increase from 2015 to 2022[35,36]–cost-effective farming practices have become a priority. While inorganic Cu sources (e.g., $CuSO_4 \cdot 5\,H_2O$ with ≥ 90% bioavailability; $CuCl_2$ at 80–85%) remain dominant in commercial feeds due to their economic feasibility[37], environmental concerns persist. Recent studies indicate elevated Cu concentrations in China's coastal waters, ranging from 0.05 to 6.77 μg/L[13], frequently exceeding the Class I seawater quality standard (≤ 5 μg/L) established by China's National Standard (GB 3097–1997)[38]. These findings underscore the critical need to precisely evaluate the nutritional copper requirements of farmed coho salmon, ensuring both optimal growth performance and environmental sustainability in intensive aquaculture systems[22,28,39,40]. The study focuses on exploring how different levels of dietary Cu impact the growth performance, Cu concentration in tissue, antioxidant capacity and hematological parameters of coho salmon. At the same time, the Cu requirement of coho salmon determined by the above indicators, which has a certain practical guiding for the production of the fish feed.

Methods and materials

Experimental diets

The formula of the diet is shown in Table 1. A purified basal diet (42.92% crude protein and 9.93% crude lipid) was formulated with six levels of Cu ($CuSO_4$ used as Cu source, Sinopharm Chemical Reagent Co., Ltd, SCR, Shanghai, China) (0.00, 2.00, 4.00, 6.00, 8.00, and 10.00 mg kg^{-1}). The measured values of dietary Cu contents were 0.20, 2.10, 3.70, 5.80, 7.75, and 9.85 mg/kg.

Fish and experimental procedures

All fish procedures were conducted in accordance with the ARRIVE guidelines (https://arriveguidelines.org) and approved by the Laboratory Animal Welfare & Ethics Committee of Weifang University (No. 20210413007) prior to the experimentation. All experimental methods were carried out in accordance with relevant guideline and regulations.

Feeding experiment was conducted in the breeding base of Shandong Wanda Fishery Co., Ltd. on February 10, 2021. The fish were provided by the Conqueren Leading Fresh (Shandong) Marine Science & Technology Inc., Ltd., China, and fed the control diet to acclimate for two weeks before the experiment began. After two weeks of acclimation, total of 180 healthy, uniformly sized experimental fish (180.22 ± 0.41 g) were selected to eighteen floating cages (1 m × 1 m × 1 m, L × W × H, 1,000-L, 10 fish/cage), which hung in a concrete culture pool at the breeding base. During the culture trial period, the fish were raised in a consistently filtered subterranean spring cold-water (13–15 ℃). Feed three times a day (8:00, 12:00, and 17:00), with the daily ratio being 3–5% of body weight and adjusted according to prior feeding responses. The experiment was lasted under natural conditions for 10 weeks.

Ingredients	Dietary Cu levels (mg/kg) 0.00	2.00	4.00	6.00	8.00	10.00
Casein	40.00	40.00	40.00	40.00	40.00	40.00
Gelatin	10.00	10.00	10.00	10.00	10.00	10.00
Refined fish oil	7.50	7.50	7.50	7.50	7.50	7.50
Soybean oil	7.50	7.50	7.50	7.50	7.50	7.50
Dextrin	16.00	16.00	16.00	16.00	16.00	16.00
α-Cellulose	9.50	9.50	9.50	9.50	9.50	9.50
Vitamin premix[1]	1.00	1.00	1.00	1.00	1.00	1.00
Mineral premix[2]	6.00	6.00	6.00	6.00	6.00	6.00
Methionine	0.50	0.50	0.50	0.50	0.50	0.50
Arginine	1.00	1.00	1.00	1.00	1.00	1.00
Antioxidant	0.60	0.60	0.60	0.60	0.60	0.60
Vc phosphate ester	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride	0.30	0.30	0.30	0.30	0.30	0.30
Betaine	0.05	0.05	0.05	0.05	0.05	0.05
$CuSO_4 \cdot 5H_2O$	0.00	8.00	16.00	24.00	32.00	40.00
Nutrient composition (% dry matter)						
Crude protein	43.06	42.91	42.84	42.91	42.82	42.96
Crude lipid	9.82	9.94	9.92	9.94	10.04	9.93
Crude ash	6.56	6.68	6.53	6.54	6.65	6.52
Moisture	7.26	7.32	7.21	7.34	7.26	7.39
Cu contents (mg/kg)	0.20	2.10	3.70	5.80	7.75	9.85

Table 1. Formulation and proximate composition of the experimental diets for Coho salmon *Oncorhynchus kisutch* (% dry matter). Note: [1]Vitamin premix (mg/kg Ingredients): Vitamin D3, 0.04; Vitamin E, 50; Vitamin K, 44.0; Vitamin B1, 12.0; Vitamin B2, 25.0; Vitamin B5, 20.0; Vitamin B6, 15.0; choline chloride, 500.0; inositol, 200.0; biotin, 0.5; folic acid, 1.5; Vitamin C, 100.0; niacin, 75.0; Vitamin B12, 0.01. [2]Mineral premix (mg/kg Ingredients): AlK $(SO_4)_2 \cdot 12H_2O$, 124.0; $CaCl_2$, 17,880.0; $CoCl_2 \cdot 6H_2O$, 49.0; $FeSO_4 \cdot 7H_2O$, 707.0; KCl, 1192.0; KI, 5.0; $MgSO_4 \cdot 7H_2O$, 4317.0; $MnSO_4 \cdot 4H_2O$, 31.0; NaCl, 4934.0; $Na_2SeO_3 \cdot H_2O$, 3.0; $ZnSO_4 \cdot 7H_2O$, 177.0; Ca $(H_2PO_4)_2 \cdot H_2O$, 12,457.0; KH_2PO_4, 9930.0.

Sampling
After the feeding trial, fast for 24 h, the fish was counted and weighed in each cage. Ten fish from each repetition for anesthetized (MS-222, 20 mg/L), in which 5 fish store at −20 ℃ for morphological indicators, proximate composition and mineral composition determination. The blood samples were drawn from the caudal vein of the remaining 5 fish after measuring their body length and weight. After standing for two hours, serum samples were collected and centrifuged at 3000 g for 15 min, and then store at −80 ℃ for the determination of total SOD (T-SOD), Cu-Zn SOD, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lysozyme (LZM), alkaline phosphatase (AKP) activities. After blood collecting, the liver sample from same repetition were separate and pooled, and then store at −80 ℃ for the follow-up analysis of hepatic T-SOD, CAT and Cu-Zn SOD activities, malondialdehyde (MDA) content.

Analytical methods
Growth methods
Quantity and weight of fish were recorded at beginning(Q_0, W_0) and termination of the experiment (Q_1, W_1), and fish length (FL, cm), feed intake (FI, g), body weight (W_b, g), liver weight (W_l, g) and intestinal weight (W_i, g) during the experiment time:

$$\text{Survival rate (SR, \%)} = 100 \times Q_1/Q_0$$

$$\text{Specific growth ratio (SGR, \%/day)} = 100 \times (\ln W_1 - \ln W_0)/70$$

$$\text{Condition factor (CF, g/cm}^3) = 100 \times W_b/FL^3$$

$$\text{Hepatosomatic index (HSI, \%)} = 100 \times W_l/W_b$$

$$\text{Viscerosomatic index (VSI, \%)} = 100 \times W_i/W_b$$

$$\text{Feed conversion ratio (FCR)} = FI/(W_1 - W_0)$$

Proximate composition analysis
The proximate composition analysis of diet and muscle were assessed by the AOAC[41] standard method. Among them, the determination of moisture was achieved by drying in a 105 ℃ oven (HWXT-9140 A, Aodema Corporation, Shenzhen, China) to a constant weight, crude protein by the Kjeldahl method (Kjeltec 8000, Foss, Denmark), crude lipid by the Soxhlet extraction method (Soxtec 2043, Foss, Denmark), ash by burned at 550 ℃ in a muffle furnace (SX2-4–12 A, Shanghai Jiecheng Corporation, Shanghai, China) for 12 h. Cu contents of diets, muscle and liver were determined by employing the inductively coupled plasma atomic emission spectrometer (ICP-OES Optima 5300DV, Perkin Elmer Corporation, USA)[42].

Liver and hematological analysis
The liver tissues were homogenized in 9 volumes (w/v) of ice-cold 0.1 M phosphate buffer (pH 7.4) containing 1.15% KCl using a homogenizer. This produced a 10% (w/v) homogenate, which was then centrifuged at 10,000 × g for 15 min at 4 °C to obtain the supernatant for subsequent analysis. Hepatic MDA content was determined by thiobarbituric acid (TBA) method at 532 nm[43]. Hepatic T-SOD and Cu-Zn SOD activities were assayed by the method of xanthine oxidase at 550 nm[44]. Hepatic CAT activity was assayed by the method of ammonium molybdate method at 405 nm[45]. Serum AKP activity was assayed by NPP substrate-AMP buffer method at 405 nm[46]. Serum LZM content was assayed by turbidimetric method at 450 nm[47]. Serum AST and ALT activities were assayed by ultraviolet colorimetric method at 340 nm[48].

Statistical analyses
The data was analyzed utilizing one-way analysis of variance (ANOVA) with SPSS version 25.0 software (SPSS Inc., Armonk, New York, USA). The data (means ± standard deviation) were compared using Duncan's multiple comparison post-hoc analysis, with statistical significance set at $P < 0.05$. Broken-line analysis was used for analyzing the optimal dietary Cu level based on SGR, liver Cu accumulation, Cu-Zn SOD in liver and serum.

Results
Growth performance
No mortality was observed in coho salmon with varying levels of dietary Cu ($P > 0.05$) (Table 2). The FBW and SGR in the control group (0.20 Cu mg/kg) were significantly lower ($P < 0.05$) than that in the other groups, FCR showed an inverse trend. The fish in 5.8 mg/kg group had the highest FBW and SGR, and the lowest FCR. However, no notable variances ($P > 0.05$) in morphological parameters (HSI, VSI and CF) of the fish fed dietary Cu levels ($P > 0.05$). As per the SGR results, the appropriate dietary Cu for coho salmon was 5.41 mg/kg (Fig. 1A).

Proximate composition and Cu concentration
The dietary Cu levels did not significantly affected proximate composition of the fish ($P > 0.05$), whereas increasing dietary Cu increased the muscle and liver Cu content of fish ($P < 0.05$) but the Cu content in muscle and liver did not further increase when dietary Cu level exceed 5.8 mg/kg (Table 3). Based on liver Cu content the appropriate dietary Cu for coho salmon was 5.29 mg/kg (Fig. 1B).

Hepatic and serum biochemical analysis
Graded dietary Cu levels have a significantly improve ($P < 0.05$) on hepatic and serum CAT, T-SOD and Cu-Zn SOD activities and inhibit hepatic MDA content in coho salmon (Table 4). In the control group (0.20 Cu mg/kg), hepatic CAT, T-SOD and Cu-Zn SOD activities, and serum T-SOD and Cu-Zn SOD activities were the lowest, and MDA content was the highest. Nevertheless, these antioxidant enzymes reaching highest with the Cu level in the diet reaches 5.80 mg/kg and higher (> 5.80 mg/kg) Cu level in diet led to decrease. The content of MDA in 5.80 mg/kg group was the lowest, which was 7.16 nmol/mg port. Based on Cu-Zn SOD activity in liver and serum showed that the appropriate dietary Cu for coho salmon were 5.92 and 5.55 mg/kg (Fig. 1C and D).

Dietary Cu levels had a significantly effect on serum ALT, AST, AKP and LZM activities in coho salmon ($P < 0.05$) (Table 5). ALT and AST activities in serum showed decreased trend with the increasing dietary Cu

	Dietary Cu levels (mg/kg)						
Ingredients	0.20	2.10	3.70	5.80	7.75	9.85	*P*
IBW (g)	185.41 ± 0.23	185.22 ± 0.30	186.05 ± 0.33	185.96 ± 0.46	186.01 ± 0.27	186.04 ± 0.38	0.413
FBW (g)	380.33 ± 3.41^{a}	396.70 ± 3.64^{b}	407.33 ± 2.55^{bc}	416.20 ± 3.19^{c}	402.53 ± 2.28^{bc}	398.67 ± 3.73^{b}	0.033
SGR (%/day)	1.27 ± 0.04^{a}	1.35 ± 0.02^{b}	1.40 ± 0.01b^{c}	1.43 ± 0.01^{c}	1.37 ± 0.01^{bc}	1.34 ± 0.02^{b}	0.031
FCR	2.32 ± 0.04^{c}	2.14 ± 0.10^{b}	2.05 ± 0.03^{ab}	1.97 ± 0.03^{a}	2.10 ± 0.04^{ab}	2.14 ± 0.04^{b}	0.029
CF	1.41 ± 0.10	1.36 ± 0.11	1.43 ± 0.04	1.31 ± 0.05	1.29 ± 0.10	1.38 ± 0.30	0.625
SR (%)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	–
HSI (%)	2.12 ± 0.24	2.06 ± 0.24	2.07 ± 0.28	2.88 ± 0.34	2.10 ± 0.13	2.10 ± 0.37	0.521
VSI (%)	6.23 ± 0.19	5.83 ± 0.29	5.89 ± 0.70	4.94 ± 0.30	5.73 ± 0.34	6.21 ± 0.38	0.632

Table 2. Growth performance and feed utilization of Coho salmon *Oncorhynchus kisutch* fed the experimental diets with different Cu levels after 10 weeks. Abbreviations: SR, Survival rate; IBW, Initial body weight; FBW, Final body weight; SGR, specific growth rate; CF, condition factor; HSI, hepatosomatic index; VSI, Viscerosomatic index; FCR, Feed conversion ratio. [a]Each value represents the mean of 3 replicates.

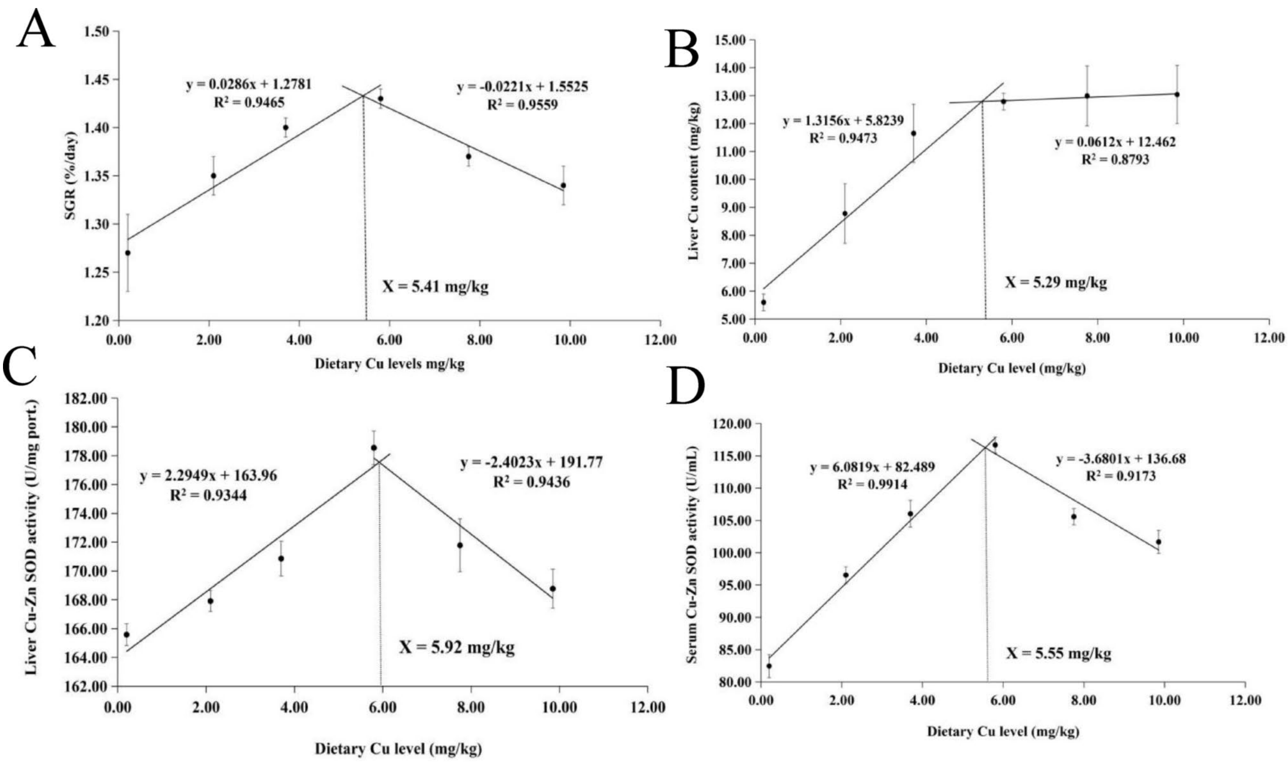

Fig. 1. Effect of dietary copper level on SGR, liver Cu content, liver and serum Cu-Zn SOD activities of coho salmon (*Oncorhynchus kisutch*) fed the experimental diets for 10 weeks. (**A**) SGR; (**B**) Liver Cu content; (**C**) liver Cu-Zn SOD activities; (**D**) serum Cu-Zn SOD activities. The optimal dietary copper requirement were 5.41 mg/kg, 5.29 mg/kg, 5.92 mg/kg and 5.55 mg/kg, respectively. Each point represents the mean of three replicates.

Ingredients	Dietary Cu level (mg/kg) 0.20	2.10	3.70	5.80	7.75	9.85	*P*
Muscle							
Moisture (%)	70.00 ± 1.31	70.19 ± 1.56	70.03 ± 3.37	70.08 ± 3.31	70.13 ± 2.73	70.81 ± 2.16	0.426
Crude protein (%)	13.03 ± 0.27	13.05 ± 0.41	13.10 ± 0.72	13.98 ± 0.79	13.93 ± 0.92	13.84 ± 0.19	0.321
Crude lipid (%)	5.69 ± 0.17	5.67 ± 0.43	5.51 ± 0.08	5.50 ± 0.09	5.49 ± 0.02	5.47 ± 0.10	0.587
Ash (%)	3.09 ± 0.18	3.09 ± 0.23	3.06 ± 1.37	3.06 ± 0.13	3.09 ± 0.13	3.03 ± 0.54	0.698
Cu (mg/kg)	1.01 ± 0.04^{a}	1.29 ± 0.03^{b}	1.45 ± 0.04^{c}	1.51 ± 0.05^{d}	1.54 ± 0.07^{d}	1.56 ± 0.12^{d}	< 0.001
Liver							
Cu (mg/kg)	5.60 ± 1.03^{a}	8.78 ± 1.02^{b}	11.65 ± 0.40^{c}	12.79 ± 0.30^{d}	12.99 ± 1.07^{d}	13.04 ± 1.04^{d}	0.001

Table 3. Muscle proximate composition of Coho salmon *Oncorhynchus kisutch* fed the experimental diets with graded Cu levels after 10 weeks. [a]Each value represents the mean of 3 replicates.

level (0.20–5.80 mg/kg), and then raised. The lowest ALT and AST activities were both found in 5.80 mg/kg group. The serum AKP and LZM activities had an opposite changing pattern with ALT and AST activities. These markedly increasing when the increase of dietary Cu level (0.20–5.80 mg/kg), and then decreasing with the increasing dietary Cu level, and both reaching the peak value in 5.80 mg/kg group.

Discussion

Cu is a vital element for vertebrates, including fish, insufficient or excessive dietary Cu supplementation is not conducive to the growth of fish[21][,37][,49,50,51]. In current research, mortality rates were found to be unaffected by dietary Cu level, these results were consistent with earlier research conducted on gilthead seabream *Sparus aurata*[14] and stinging catfish *Heteropneustes fossilis*[52]. However, it was observed that dietary Cu supplementation positively influenced the growth performance of coho salmon, in terms of FBW, SGR and FCR. Coho salmon with higher Cu diet (3.70–7.75 mg/kg) obtained improved fish growth and lower FCR, but the lowest FBW and SGR and the highest FCR were observed fish fed the diet without Cu supplementation diet (0.20 mg Cu/kg).

Ingredients	Dietary Cu level (mg/kg)						P-value
	0.20	2.10	3.70	5.80	7.75	9.85	
Liver							
T-SOD	185.45 ± 0.81[a]	198.63 ± 0.86[b]	210.90 ± 1.29[c]	237.92 ± 1.20[d]	215.09 ± 3.76[c]	201.95 ± 1.23[b]	< 0.001
Cu-Zn SOD (U/mg prot.)	165.58 ± 0.77[a]	167.91 ± 0.72[ab]	170.86 ± 1.21[b]	178.55 ± 1.17[c]	171.78 ± 1.84[b]	168.77 ± 1.35[ab]	< 0.001
CAT (U/mg prot.)	34.21 ± 0.25[a]	39.42 ± 0.15[b]	45.80 ± 0.25[c]	48.80 ± 0.02[d]	46.13 ± 0.24[c]	40.12 ± 0.50[b]	< 0.001
MDA (nmol/mg prot.)	8.64 ± 0.05[d]	8.07 ± 0.09[c]	7.48 ± 0.14[b]	7.16 ± 0.08[a]	7.42 ± 0.08[ab]	8.17 ± 0.02[c]	< 0.001
Serum							
T-SOD (U/mL)	115.60 ± 1.23[a]	121.88 ± 1.72[b]	140.80 ± 1.80[c]	147.88 ± 1.23[d]	136.78 ± 2.39[c]	121.77 ± 1.88[b]	< 0.001
Cu-Zn SOD (U/mL)	82.47 ± 1.80[a]	96.54 ± 1.30[b]	106.03 ± 2.09[c]	116.68 ± 1.23[d]	105.58 ± 1.24[c]	101.68 ± 1.78[c]	< 0.001

Table 4. Anti-oxidative enzymes activities in Coho salmon *Oncorhynchus kisutch* fed the experimental diets with graded Cu levels after 10 weeks[a]. Abbreviations: U/mg port.:Units per milligram of protein. [a]Each value represents the mean of 3 replicates.

Ingredients	Dietary Cu level (mg/kg)						P-value
	0.20	2.10	3.70	5.80	7.75	9.85	
AST (U/L)	7.88 ± 0.01[d]	6.05 ± 0.05[c]	4.19 ± 0.03[b]	3.61 ± 0.02[a]	4.13 ± 0.02[b]	5.02 ± 0.32[c]	< 0.001
ALT (U/L)	5.98 ± 0.03[d]	4.99 ± 0.03[c]	3.42 ± 0.01[b]	3.04 ± 0.06[a]	3.37 ± 0.05[b]	4.96 ± 0.02[c]	< 0.001
AKP (U/mL)	0.78 ± 0.00[a]	0.85 ± 0.00[b]	1.01 ± 0.00[c]	1.13 ± 0.00[d]	1.02 ± 0.00[c]	1.04 ± 0.00[c]	< 0.001
LZM (μg/mL)	4.86 ± 0.01[a]	5.29 ± 0.02[b]	6.01 ± 0.01[c]	6.19 ± 0.04[d]	5.98 ± 0.01[c]	5.23 ± 0.01[b]	< 0.001

Table 5. Serum biochemical and immunity parameters in Coho salmon *Oncorhynchus kisutch* fed the experimental diets with graded Cu levels after 10 weeks[a]. Abbreviations: Abbreviations: AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; AKP: alkaline phosphatase; LZM: lysozyme. [a]Each value represents the mean of 3 replicates.

Chen et al.[53] found that compared to the adequate Cu (4.18 mg/kg) diet, Cu deficiency (0.76 mg/kg) and excess (92.45 mg/kg) diet can lead to reduce growth rate, the weight gain (WG) and SGR in yellow catfish *Pelteobagrus fulvidraco*. Comparable findings have indicated that the growth performance of Russian sturgeon *Acipenser gueldenstaedtii*[54] can be impacted by dietary Cu, indicating that supplementation Cu to fish is necessary, and more importantly thing is to find the proper equilibrium between meeting the nutritional needs of fish while preventing its potential toxicity[39]. According to the broken-line regression finding of SGR (Fig. 1A), the ideal dietary Cu requirements for coho salmon was found to be 5.41 mg/kg, mirroring the outcomes of earlier research conducted on tilapia *Oreochromis niloticus 5 O. aureus*(4 mg Cu/kg)[55] and stinging catfish (5.24–5.68 mg/kg)[52]. However, these findings are quite different from those of gilthead seabream(*Sparus aurata*) larvae (21.5–22.6 mg/kg)[14], which is suggested that the possible causes may be due to different fish species, experimental conditions[22] and experimental feed formulations[14[,28]. Compared with the 5.80 Cu mg/kg in diet, although 7.75 Cu mg/kg level lower growth performance but no significant difference, while higher dietary Cu level (9.85 mg/kg) was slightly reduced than the above two groups. This suggests that when dietary Cu levels are above 5.80 mg/kg, the growth performance has an inflection point, while more than 9.85 mg/kg, there may be toxic effects on coho salmon. As Chen et al.[53] and Kim and Kang[56] suggest, the energy available allocation to growth is reduced in order to maintain the detoxification of excess metals, leading to a decline in growth performance, and the feed efficiency is adversely affected.

Fish morphological parameters usually refer to a quantitative index obtained by directly measuring the external and internal structure of fish. External morphological parameters include CF, length-to-increase ratio, etc., and internal organ proportion parameters include HSI, VSI, gonadalsomatic index (GSI), etc[57]. The findings of the research indicated that the morphological parameters of coho salmon remained unaffected by dietary Cu level, including CF, HSI and VSI. Likewise, Cao et al.[58] and El Basuini et al.[59] observed no significant impact of dietary Cu levels on the morphological parameters of large yellow croaker *Larimichthys croceus* and red sea bream *Pagrus major*. Unlike growth, CF parameters of Atlantic salmon fry were less affected by dietary Cu[60]. No significantly effect on the morphological parameters could be explained by the fact that insufficient or excessive dietary Cu supplementation did not affect proximate composition in muscle. Similar findings were observed in juvenile larger yellow croaker *Larimichthys croceus*[20] and gilthead seabream[14]. However, El Basuini et al.[27] found that fish fed 2 mg/kg nanocopper (Cu-NPs) diet had higher crude protein in the whole body compared with 0 mg/kg diet. Whole body lipid content also decreased as Cu levels in the diet increased in previous studies[20[,61[,62]. In this study, muscle protein and lipid had a bit vary tendency but not significant, which seems to indicate that the deposition of protein and lipids in relation to Cu supplementation is dependent on both the dietary Cu concentration and form of Cu supplementation, and also tissue specificity.

Excessive Cu deposition in tissues can cause poisoning and damage to several organs, including liver and gill[25]. The liver serves as the primary storage site for Cu in fish[27[,61]. The muscle and liver Cu content of coho salmon showed a positive correlation between dietary Cu level, and the Cu content of tissue in high dietary

Cu level (9.85 mg/kg) was higher than the non-supplemented diet (0.20 Cu mg/kg). The plateau in tissue copper deposition (both hepatic and muscular) at dietary concentrations between 5.8 and 9.6 mg/kg suggests the existence of homeostatic regulation mechanisms that limit further Cu accumulation beyond this range. Compared to other organs, Cu deposition and homeostasis are mainly in the liver[52,63]. In a study by Liang et al.[28], blunt snout bream fed a diet containing 9.13 mg/kg of Cu for 10 weeks exhibited a 16.4% increase in whole body Cu content compared to the control group. High Cu intake can contribute to approximately 99% of the total body Cu content in fish[10]. Consistent with grouper *Epinephelus malabaricus*[64] and beluga, *Huso huso*[61]. Importantly, higher dietary Cu levels (9.85 mg/kg) in coho salmon significantly retarded growth and weaken antioxidant capacity. The observed plateau in tissue Cu deposition at higher dietary levels likely represents a protective mechanism against Cu overload, though this regulatory capacity appears insufficient to prevent adverse symptoms when dietary copper exceeds the optimal range.

Cu serves as a co-factor in various cellular antioxidant defense mechanisms, such as SOD, Cu-Zn SOD, ceruloplasmin, in addition, the system also includes CAT, GPx and so on[23,25,54]. These antioxidant enzymes removal of induced ROSs which are highly toxic to organisms by catalytic dismutase of superoxide radicals[9,64–66]. Dietary Cu regulates the antioxidant response by regulating antioxidation-related enzymes activities had been proved in previous fish studies[14,25,28,29,67]. In hybrid tilapia *Oreochromis niloticus* × *Oreochromis aureus*, optimal Cu supplementation led to a significant rise in the activities of Cu-Zn SOD and SOD[68]. Hepatic T-SOD and Cu-Zn SOD activities in blunt snout bream were enhanced by a dietary intake of 5.21–9.13 mg/kg Cu compared to a diet without supplementation[28]. The results of the study indicated that the antioxidant status of coho salmon was positively impacted by the right amount of Cu supplementation in their diet. Inhibited SOD and Cu-Zn SOD activities in serum and liver when dietary Cu supplementation was insufficient (0.20–2.10 mg/kg) or excessive (7.75–9.85 mg/kg). These results confirm that appropriate dietary Cu supplementation can enhance the antioxidant defense by improving the production of Cu-Zn SOD and SOD, thus lowering the susceptibility to oxidative damage[69]. Furthermore, hepatic CAT activity was the highest when dietary Cu supplementation was 5.80 mg/kg, which was consistent with the study by Zafar and Khan[52] that lower (3.65 to 4.63 mg/kg) or higher (6.19 to 6.69 mg/kg) dietary Cu level were significantly suppressed Cu-Zn SOD and CAT activities than optimum (5.28 mg/kg) level in stinging catfish *Heteropneustes fossilis*. These results demonstrate that insufficient or excessive dietary Cu supplementation could decreased related metabolic enzymes activities and increase the oxidative damage of ROSs[61,62]. Compared with Cu deficiency depresses antioxidant enzyme activity, Cu overload can increase oxidative damage[25]. It is mainly due to the toxic effects caused by free radicals produced by Cu accumulates in the tissues[9,14,70]. MDA, a product of lipid per-oxidation caused by ROSs oxidative damage, which can directly reflect the strength of lipid per-oxidation in vivo[68,71]. In this study, hepatic MDA content was higher in non-supplemented (0.20 Cu mg/kg) and 9.85 Cu mg/kg diet, which was consistent with findings that elevated levels of hepatic thiobarbituric acid reactive substances (TBARS) in lower (3.65 to 4.63 mg/kg) or higher (6.19 to 6.69 mg/kg) dietary Cu level in stinging catfish[52]. In addition, Liang et al.[28] reported that appropriate dietary Cu supplementation can also reduce plasma MDA content. In summary, the appropriate dietary Cu supplementation has the potential to improve the antioxidant capacity of coho salmon, thereby reducing oxidative stress risk.

Both AST and ALT are commonly utilized as biomarkers to assess liver damage[56,72]. Research has indicated that serum ALT and AST increased of fish usually were infected by toxins, virus and bacteria, malnutrition or damage of liver, which is accompanied by the release of transaminases into the circulation[61,66,73]. In a study by Liang, et al.[28], dietary supplementation with lower (1.43 mg/kg) and higher (9.13 mg/kg) levels of Cu significantly elevated the ALT levels in plasma compared to snout bream fed with 5.21 mg/kg of dietary Cu. Serum ALT and AST activities decreased with the increase in dietary Cu in hybrid tilapia[66]. The study also showed a significant reduction in serum ALT and AST activities in coho salmon fed with a diet containing 5.80 mg/kg of Cu, indicating that the liver was in a healthy state.

Blood parameters serve as critical biomarkers for evaluating both antioxidant capacity and immune competence in fish[74]. Non-specific immunity is crucial in providing early defense for bony fish against pathogens, as specific immunity is restricted by environmental conditions[75]. In the non-specific immune system, AKP is a non-specific immune marker enzyme in fish, which can effectively reflect the defense ability against exogenous substance of organism[76,77]. Post-smolt serum AKP levels were elevated with rising dietary Cu concentrations, but decreased when Cu levels exceeded 5.80 mg/kg. Shao et al.[78] also found that the AKP activity of the fish was correlated with the level of Cu in diets, and low Cu significantly reduced that enzyme activity. Suitable dietary Cu can activate the serum AKP activity, but with the concentration exceeding the tolerated range of organisms, the activity of AKP will be significantly inhibited, thus inhibiting their non-specific immunity[79]. LZM is a mucolytic enzyme of fish, which belongs to the non-specific immune. It has antibacterial activity and can increase innate resistance to bacteria and viral[22,80]. Jin et al.[22] confirmed that serum LZM activity significantly decreased when yellowtail kingfish exposed to Cu concentrations ≥ 0.12 mg/L. Dietary Cu has been shown to impact serum LZM levels and immune-related gene expression in hybrid tilapia[66]. The research also discovered that appropriate dietary Cu levels can enhance the serum LZM activity in coho salmon, while low or high dietary Cu can induce immunosuppression by decreasing the LZM[80]. In other words, these findings highlight the importance of dietary Cu in modulating the immune responses of fish and suggest potential strategies for improving their resistance to pathogens.

Conclusion

In general, the dietary Cu level has a great effect on the growth and physiological function of coho salmon. Suitable dietary Cu can improve FBW and SGR, reduce FCR, depresses the ALT and AST activities, activate and regulate antioxidant capacity and immunity, as well as higher dietary Cu (9.85 mg/kg) has starting to show signs of toxicity for coho salmon. Based on the SGR, liver Cu content, liver and serum Cu-Zn SOD activities, the appropriate dietary Cu supplementation range of coho salmon were estimated to be 5.29–5.92 mg/kg.

Data availability

Data will be made available on request. All the data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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