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Short communication

Dietary copper requirements of juvenile large yellow croaker *Larimichthys croceus*

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ABSTRACT

A 10-week feeding trial was conducted to estimate the requirement of dietary copper for juvenile large yellow croaker *Larimichthys croceus* (initial body weight, 9.18 ± 0.06 g). Six graded levels of dietary copper were designed as 2.61, 3.25, 4.65, 7.16, 11.38, and 18.45 mg kg^{-1}, respectively. Results showed that fish fed the basal diet with 2.61 mg kg^{-1} of copper had the significantly lowest weight gain rate (WGR), activity of copper-zinc superoxide dismutase (Cu-Zn SOD), the total antioxidant capacity (T-AOC), the whole-body Cu concentration and the vertebrae Cu concentration ($P < 0.05$). When dietary copper increased from 2.61 to 4.65 mg kg^{-1}, the WGR significantly increased ($P < 0.05$). Higher dietary copper contents did not result in further increasing of WGR. Higher (>7.16 mg kg^{-1}) dietary copper content significantly decreased the Cu-Zn SOD activity and T-AOC in liver ($P < 0.05$). No significant differences were found in survival, feed efficiency, body compositions, hepatosomatic index, viscerosomatic index and condition factor among the all treatments ($P > 0.05$). Based on the WGR, the optimal dietary copper content for large yellow croaker was estimated to be 3.41 mg kg^{-1}. Based on the whole-body Cu concentration, the vertebrae Cu concentration and the Cu-Zn SOD activity in serum, the minimum dietary copper content was estimated to be 5.30, 5.90 and 7.05 mg kg^{-1}, respectively.

1. Introduction

Copper (Cu) is an essential trace element for all animals including fish. It plays a variety of biological functions (Davis and Mertz, 1987; Lall, 2002). Copper is a vital component of several enzymes (e.g., superoxide dismutase and cytochrome oxidase) that are involved in oxidation–reduction reactions and occurs tightly bound to proteins in the cell rather than as free ions. It also plays an important role in brain neurotransmitters and collagen synthesis (Halver and Hardy, 2002).

Although fish can absorb minerals from environment, diet is considered to be the major source of minerals for fish (Watanabe et al., 1997). Copper deficiency slowed down the growth of carp *Cyprinus carpio* (Ogino and Yang, 1980) and decreased the activities of heart cytochrome c oxidase and liver copper-zinc superoxide dismutase (Cu-Zn SOD) in channel catfish *Ictalurus punctatus* (Gatlin and Wilson, 1986). However, excessive dietary Cu can reduce growth and feed conversion efficiency (FCE) in channel catfish (Murai et al., 1981) and rainbow trout *Salmo gairdneri Richardson* (Lanno et al., 1985). Hence, an optimal dietary copper level is important for aquatic animals. The quantity requirement of dietary copper has been established for many fish species, such as rainbow trout and carp (Ogino and Yang, 1980), channel catfish (Gatlin and Wilson, 1986), Atlantic salmon *Salmo salar* (Lall and Hines, 1987; Lorentzen et al., 1998), yellow catfish *Pelteobagrus fulvidraco* (Tan et al., 2011) and grass carp *Ctenopharyngodon idella* (Tang et al., 2013).

Large yellow croaker is a commercially important marine species and widely cultured in China. The production of the cultured large yellow croaker in 2011 was more than 80,000 tons in China (China Fishery Statistical Yearbook, 2012). Dietary requirements of most nutrients have been established for this species in the last decades. In regard to the mineral requirements, the optimal dietary levels of phosphorus, iron and zinc were determined as 0.89–0.91% (Mai et al., 2006), 101.2 mg kg^{-1} (Zhang, 2007) and 59.6–84.6 mg kg^{-1} (Zhang et al., 2008), respectively. The present study was designed to determine the requirement of dietary copper for juvenile large yellow croaker.

2. Materials and methods

2.1. Experimental diets

The formulation and proximate composition of the basal diet are presented in Table 1. Casein, gelatin and fish muscle protein were used as the dietary protein sources. Fish oil was used as the main dietary lipid source. Dextrin was used as the carbohydrate source. Isonitrogenous (48.77% of crude protein) and isolipidic (10.08% of crude

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Table 1
Formulation and proximate composition of the experimental diets (% dry matter).

Ingredient	Diet number					
	Diet-1	Diet-2	Diet-3	Diet-4	Diet-5	Diet-6
Casein, vitamin-free[a]	30	30	30	30	30	30
Gelatin[b]	8	8	8	8	8	8
Fish muscle protein[c]	9	9	9	9	9	9
Dextrin[d]	25	25	25	25	25	25
Fish oil[e]	9	9	9	9	9	9
Lecithin	3	3	3	3	3	3
Vitamin premix[f]	2	2	2	2	2	2
Mineral premix, copper-free[g]	1	1	1	1	1	1
Attractant	1	1	1	1	1	1
Taurine	0.5	0.5	0.5	0.5	0.5	0.5
Ethoxyquin	0.05	0.05	0.05	0.05	0.05	0.05
Mold inhibitor	0.1	0.1	0.1	0.1	0.1	0.1
Monocalcium phosphate	1	1	1	1	1	1
Cellulose	10.35	10.35	10.35	10.35	10.35	10.35
Proximate composition ($n = 3$)						
Crude protein (%)	46.8	46.9	46.1	46.5	47.1	47.2
Crude lipid (%)	10.0	9.7	10.0	10.1	9.8	9.9
Moisture (%)	7.6	7.6	8.0	8.0	7.9	7.8
Ash (%)	2.2	2.3	2.1	2.1	2.2	2.1
Copper (mg kg^{-1})	2.61	3.25	4.65	7.16	11.38	18.45

[a] Casein, vitamin-free: crude protein 92.24%, crude lipid 0.84% (Sigma Chemical, St. Louis, MO, USA).
[b] Gelatin: Shandong Yixin Biological Technology Co., Ltd., Shandong Province, China.
[c] Fish muscle protein: Shanghai Hai Qing Aquatic Bio-tech. Co., Ltd., Shanghai, China.
[d] Dextrin: Shandong Xiwang Sugar Co., Ltd., Shandong Province, China.
[e] Fish oil: Qingdao Great-seven Bio-tech. Co., Ltd., Qingdao, China.
[f] Vitamin premix (mg kg^{-1} diet): retinol acetate, 32; cholecalciferol, 5; menadione sodium bisulfite, 5.1; α-tocopherol, 120; thiamin-HCl, 25; riboflavin, 36.7; pyridoxine-HCl, 20; vitamin B12, 0.1; D-pantothenic acid calcium, 60; niacin acid, 200; folic acid, 20; biotin, 1.2; inositol, 792; ascorbic acid, 2000; choline chloride, 4000; cellulose, 12,683.
[g] Mineral premix, copper-free (mg kg^{-1} diet): $MgSO_4 \cdot 7H_2O$, 1826; $FeSO_4 \cdot 7H_2O$, 119; $ZnSO_4 \cdot 7H_2O$, 76; $MnSO_4 \cdot H_2O$, 44; $CoCl_2 \cdot 6H_2O$, 2; Na_2SeO_3, 0.45; $Ca(IO_3)_2 \cdot 6H_2O$, 2.35, cellulose, 7920.

lipid) semi-purified diets were formulated to contain 6 graded supplemented levels of copper (0, 1, 2, 4, 8 and 16 mg kg^{-1}, respectively). The $CuSO_4 \cdot 5H_2O$ (Sinopharm Chemical Reagent Co., Ltd, SCR, Shanghai, China) was used as the dietary copper source. Final dietary Cu concentrations were 2.61, 3.25, 4.65, 7.16, 11.38, and 18.45 mg kg^{-1}, respectively, as analyzed by the inductively coupled plasma-atomic emission spectrophotometer (ICP-OES; VISTA-MPX, VARIAN) (Tan and Mai, 2001).

2.2. Feeding trial

Large yellow croaker juveniles were obtained from a commercial farm in Ningde, Fujian province, China. Prior to initiation of the feeding trial, all the animals were reared in floating sea cages (3.0 × 3.0 × 3.0 m), and fed the basal diet for 2 weeks to adapt to the experimental diet and culturing environment.

At the start of the feeding trial, fish were not fed for 24 h. The similar size (mean initial weight: 9.18 ± 0.06 g) of healthy juveniles were chosen and randomly assigned to 18 cages (1.5 × 1.5 × 2.0 m) at density of 60 fish per cage. Each diet was fed to triplicate groups. The fish were fed to apparent satiation twice daily at 05:00 and 17:30, respectively, for 10 weeks. During the feeding trial, the water temperature ranged from 22 °C to 29.5 °C, salinity 25–28‰ and dissolved oxygen concentration was about 7 mg L^{-1}. The Cu concentration in seawater was 7.2–8.8 μg L^{-1}.

2.3. Sample collection and chemical analysis

At the end of the feeding trial, animals were not fed for 24 h. They were counted and weighted to calculate the body weight gain rate (WGR) and survival rate (SR). After that, five fish per cage were randomly selected for the determination of the whole-body composition and Cu concentration. Contents of the moisture, crude protein, crude lipid and ash in the whole-body were analyzed using the standard procedures (AOAC, 1995). Another 12 fish per cage were randomly selected to collect serum sample to analyze the Cu-Zn superoxide dismutase (Cu-Zn SOD) activity and the total antioxidant capacity (T-AOC). After that, the fish were used to calculate the hepatosomatic index (HSI) and viscerosomatic index (VSI). Six fish per cage were randomly selected to determining the vertebra Cu concentration. Another 6 fish per cage were randomly chosen to analyze the Cu-Zn SOD activity and the T-AOC in liver. The Cu-Zn SOD activity and T-AOC were measured by the commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Copper concentrations in the whole body and vertebra were determined by the inductively coupled plasma-atomic emission spectrophotometer (ICP-OES; VISTA-MPX, VARIAN).

2.4. Calculations and statistical analysis

The calculation formulae for the parameters mentioned above are as follows:

Weight gain rate (WGR, %) = 100 × [(final body weight − initial body weight)/initial body weight].
Survival (%) 100 × (final amount of fish)/(initial amount of fish).
Feed efficiency (FE) (final body weight − initial body weight)/feed intake.
Hepatosomatic index (HSI, %) (hepatic weight/body weight) × 100.
Viscerosomatic index (VSI, %) (viscera weight/body weight) × 100.
Condition factor (CF) (body weight/body length^3) × 100.

The results were presented as means ± SE of three replicates. Data were analyzed by the one-way analysis of variance (ANOVA). When overall differences were significant at less than 0.05, Tukey's test was used to compare the mean values between individual treatments.

Table 2
Survival and growth of large yellow croaker fed the experimental diets with different levels of copper for 10 weeks (means ± SE, n = 3).

Dietary Cu levels (mg kg^{-1})	IBW[1](g)	FBW[2](g)	WGR[3](%)	Survival (%)	FE [4](%)
2.61	9.18 ± 0.02	30.42 ± 0.71[a]	231.4 ± 13.6[a]	78.6 ± 4.6	1.3 ± 0.0
3.25	9.13 ± 0.02	32.77 ± 0.26[ab]	258.9 ± 3.4[ab]	77.6 ± 2.1	1.2 ± 0.0
4.65	9.16 ± 0.01	34.18 ± 0.65[b]	273.0 ± 12.7[b]	79.1 ± 4.2	1.2 ± 0.0
7.16	9.13 ± 0.02	33.51 ± 0.75[b]	267.0 ± 13.6[b]	77.6 ± 2.5	1.3 ± 0.0
11.38	9.18 ± 0.02	33.19 ± 0.64[ab]	261.6 ± 13.2[ab]	77.1 ± 3.8	1.3 ± 0.0
18.45	9.16 ± 0.04	32.99 ± 0.77[ab]	260.3 ± 14.1[ab]	81.9 ± 3.3	1.3 ± 0.0
One-way ANOVA					
F value	0.690	3.896	4.067	0.242	0.799
P value	0.641	0.025	0.022	0.936	0.588

Values in the same column sharing a common superscript letter were not significantly different (P > 0.05).
[1] IBW: Initial body weight.
[2] FBW: Final body weight.
[3] WGR: Weight gain rate.
[4] FE: Feed efficiency ratio.