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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.

**Table 3**
The whole-body compositions of juvenile large yellow croaker fed the experimental diets with different levels of copper for 10 weeks (means ± SE, n = 3).

| Dietary Cu levels (mg $kg^{-1}$) | Moisture (%) | Crude protein (%) | Crude lipid (%) | Ash (%) |
|---|---|---|---|---|
| 2.61 | 73.6 ± 0.2 | 16.6 ± 0.2 | 7.8 ± 0.2 | 3.5 ± 0.0 |
| 3.25 | 72.2 ± 0.4 | 17.0 ± 0.2 | 8.7 ± 0.2 | 3.5 ± 0.1 |
| 4.65 | 73.0 ± 0.3 | 16.6 ± 0.1 | 8.4 ± 0.2 | 3.5 ± 0.1 |
| 7.16 | 72.5 ± 0.6 | 16.8 ± 0.1 | 8.7 ± 0.3 | 3.6 ± 0.0 |
| 11.38 | 73.1 ± 0.6 | 16.7 ± 0.1 | 8.1 ± 0.4 | 3.6 ± 0.0 |
| 18.45 | 73.5 ± 0.5 | 16.4 ± 0.1 | 8.0 ± 0.4 | 3.6 ± 0.0 |
| *One-way ANOVA* | | | | |
| F value | 1.428 | 2.051 | 1.791 | 0.911 |
| P value | 0.243 | 0.100 | 0.145 | 0.487 |

Values in the same column sharing a common superscript letter were not significantly different (P > 0.05).

Statistical analysis was performed using the SPSS 17.0. The optimal dietary copper contents based on WGR, whole-body and vertebrae Cu concentration were estimated using the broken-line model (Robbins et al., 1979). For analysis of the serum Cu-Zn SOD activity, the polynomial regression was used (Zeitoun et al., 1976).

## 3. Results

### 3.1. Growth and feed utilization

The growth and feed utilization data are presented in Table 2. There were significant differences (P < 0.05) in weight gain rate (WGR) and final body weight (FBW). Fish fed the basal diet showed the significantly lowest WGR and FBW. Then WGR and FBW significantly increased as dietary copper increasing from 2.61 to 4.65 mg $kg^{-1}$ (P < 0.05), thereafter there is no further increase.

Survival and feed efficiency (FE) was not significantly affected by dietary copper levels (P > 0.05). They ranged from 77.1% to 81.9%, 1.7% to 1.9%, respectively.

### 3.2. Body composition

The body compositions are listed in Table 3. There were no significant differences in contents of moisture, crude protein, crude lipid and ash among the all treatments (P > 0.05). These contents ranged from 72.2% to 73.6%, 16.4% to 17.0%, 7.8% to 8.7% and 3.5% to 3.6%, respectively.

### 3.3. Body indices

There were no significant differences in body indices among the all treatments (P > 0.05) (Table 4). Hepatosomatic index (HSI), viscerosomatic index (VSI) and condition factor (CF) ranged from 1.4% to 1.5%, 3.4% to 3.8%, 1.7 to 1.8, respectively.

**Table 4**
The body indices of juvenile large yellow croaker fed the experimental diets with different levels of copper for 10 weeks (means ± SE, n = 3).

| Dietary Cu levels (mg $kg^{-1}$) | HSI[1] | VSI[2] | CF[3] |
|---|---|---|---|
| 2.61 | 1.4 ± 0.1 | 3.6 ± 0.2 | 1.7 ± 0.0 |
| 3.25 | 1.4 ± 0.1 | 3.6 ± 0.1 | 1.8 ± 0.0 |
| 4.65 | 1.4 ± 0.1 | 3.3 ± 0.1 | 1.4 ± 0.0 |
| 7.16 | 1.5 ± 0.1 | 3.8 ± 0.1 | 1.8 ± 0.0 |
| 11.38 | 1.4 ± 0.1 | 3.7 ± 0.1 | 1.7 ± 0.0 |
| 18.45 | 1.4 ± 0.0 | 3.7 ± 0.2 | 1.7 ± 0.0 |
| *One-way ANOVA* | | | |
| F value | 0.807 | 0.302 | 0.240 |
| P value | 0.456 | 1.231 | 1.363 |

Values in the same column sharing a common superscript letter were not significantly different (P > 0.05).
[1] HSI: hepatosomatic index.
[2] VSI: viscerosomatic index.
[3] CF: condition factor.

### 3.4. Serum and liver parameters

The results of serum and liver parameters are presented in Table 5. The significantly lowest Cu-Zn SOD activities both in serum and liver were found in the control with 2.61 mg $kg^{-1}$ of dietary copper (P < 0.05). Increasing dietary copper contents (from 2.61 to 7.16 mg $kg^{-1}$) resulted in significantly increased Cu-Zn SOD activity. Higher (>11.38 mg $kg^{-1}$) dietary copper contents led to the decrease of this activity.

The serum T-AOC had a similar changing pattern with the serum Cu-Zn SOD activity. It increased significantly when dietary copper contents were increased from 2.61 to 7.16 mg $kg^{-1}$ (P < 0.05), and then leveled off. The significantly lowest liver T-AOC was found in the treatment with 18.45 mg $kg^{-1}$ of dietary copper (P < 0.05). Increasing dietary copper levels (from 2.61 to 4.65 mg $kg^{-1}$) resulted in significantly increased liver T-AOC.

### 3.5. Copper concentration

Copper concentrations in the whole-body significantly increased with dietary Cu contents ranging from 2.61 to 7.16 mg $kg^{-1}$ (P < 0.05) (Table 6). Higher (>7.16 mg $kg^{-1}$) dietary Cu contents did not result in further increases of the whole-body Cu concentration. Similarly, the vertebrae Cu concentration significantly increased with dietary copper contents ranging from 2.61 to 11.38 mg $kg^{-1}$ (P < 0.05). When dietary copper contents increased from 11.38 to 18.45 mg $kg^{-1}$, there were no more increasing of the vertebrae Cu concentrations (Table 6).

### 3.6. Dietary copper requirement analyses

Analysis of the broken-line regression for WGR indicated that the optimal dietary copper content was 3.41 mg $kg^{-1}$ (Fig. 1). Moreover, based on serum Cu-Zn SOD activity, whole-body Cu concentration and vertebrae Cu concentration, the minimum requirement of dietary

**Table 5**
Activity of the Cu-Zn superoxide dismutase (Cu-Zn SOD) and the total antioxidant capacity (T-AOC) in serum and liver of juvenile large yellow croaker fed the experimental diets with different levels of copper for 10 weeks (means ± SE, n = 3).

| Dietary Cu levels (mg $kg^{-1}$) | Cu-Zn SOD | | T-AOC | |
|---|---|---|---|---|
| | Serum (U/ml serum) | Liver (U/mg prot.) | Serum (U/ml serum) | Liver (U/mg prot.) |
| 2.61 | $104.4 \pm 1.2^{a}$ | $57.5 \pm 1.2^{a}$ | $2.4 \pm 0.1^{a}$ | $0.4 \pm 0.0^{bc}$ |
| 3.25 | $107.1 \pm 1.2^{b}$ | $63.1 \pm 1.6^{b}$ | $2.5 \pm 0.3^{a}$ | $0.4 \pm 0.0^{c}$ |
| 4.65 | $112.7 \pm 0.1^{c}$ | $69.5 \pm 0.8^{c}$ | $2.6 \pm 0.3^{ab}$ | $0.5 \pm 0.0^{c}$ |
| 7.16 | $127.7 \pm 1.5^{d}$ | $61.7 \pm 0.6^{ab}$ | $3.6 \pm 0.1^{b}$ | $0.3 \pm 0.0^{ab}$ |
| 11.38 | $111.8 \pm 0.8^{c}$ | $60.6 \pm 0.9^{ab}$ | $2.7 \pm 0.1^{ab}$ | $0.3 \pm 0.0^{a}$ |
| 18.45 | $106.1 \pm 1.4^{ab}$ | $60.9 \pm 1.4^{ab}$ | $2.0 \pm 0.1^{a}$ | $0.3 \pm 0.0^{a}$ |
| *One-way ANOVA* | | | | |
| F value | 318.120 | 46.234 | 6.424 | 14.151 |
| P value | <0.001 | <0.001 | <0.001 | <0.001 |

Values in the same column sharing a common superscript letter were not significantly different (P > 0.05).

**Table 6**
The whole-body and vertebrae Cu concentration of juvenile large yellow croaker fed the experimental diets with different levels of copper for 10 weeks (means ± SE, n = 3).

| Dietary Cu levels (mg $kg^{-1}$) | Whole-body Cu concentration (μg $g^{-1}$) | Vertebrae Cu concentration (μg $g^{-1}$) |
|---|---|---|
| 2.61 | 5.5 ± 0.7[a] | 4.8 ± 0.2[a] |
| 3.25 | 6.2 ± 0.5[ab] | 6.5 ± 0.6[ab] |
| 4.65 | 8.8 ± 0.8[bc] | 9.2 ± 0.5[bc] |
| 7.16 | 9.1 ± 0.1[c] | 11.1 ± 0.5[cd] |
| 11.38 | 10.2 ± 0.7[c] | 12.2 ± 0.4[d] |
| 18.45 | 10.2 ± 0.4[c] | 12.6 ± 1.9[d] |
| *One-way ANOVA* | | |
| F value | 12.334 | 23.183 |
| P value | <0.001 | <0.001 |

Values in the same column sharing a common superscript letter were not significantly different (P > 0.05).

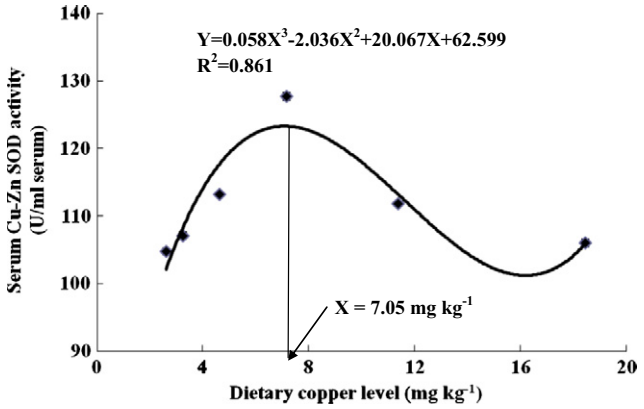


**Fig. 2.** Polynomial regression analysis of the relationship between dietary copper level and serum Cu-Zn SOD activity indicates that the minimum dietary copper content was 7.05 mg $kg^{-1}$. Each point represents the mean of three replicates. The cubic curve model was $Y = aX^3 + bX^2 + cX + d$. The 95% confidence intervals for regression equation coefficient are −0.06 to 0.17, −5.62 to 1.55, −11.19 to 51.32, −9.81 to 135.01, respectively.

copper was estimated to be 7.05 (Fig. 2), 5.30 (Fig. 3) and 5.90 (Fig. 4) mg $kg^{-1}$, respectively.

## 4. Discussion

Based on the WGR, in the present study, the optimal dietary Cu content for the juvenile large yellow croaker was estimated to be 3.41 mg $kg^{-1}$ (Fig. 1). This is similar to those in the previous studies. They were 3 mg $kg^{-1}$ for rainbow trout and carp (Ogino and Yang, 1980), 4 mg $kg^{-1}$ for hybrid tilapia *Oreochromis niloticus* × *Oreochromis aureus* (Shiau and Ning, 2003), and 3.13 mg $kg^{-1}$ for yellow catfish (Tan et al., 2011), respectively. However, it is lower than those for cobia *Rachycentron canadum* (11.5 mg $kg^{-1}$) (Qiao, 2007), grouper *Epinephelus malabaricus* (5.36 mg $kg^{-1}$) (Lin et al., 2008), spotted steed *Hemibarbus maculatus* Bleeker (8 mg $kg^{-1}$) (Lu, 2009), and grass carp (4.78 mg $kg^{-1}$) (Tang et al., 2013). Actually, the dietary Cu requirement for fish was related to the species, feeding regime, life stage, environmental conditions, and method of data analysis (Clearwater et al., 2002).

The contents of Cu in the whole body and vertebrae, in the present study, increased with dietary Cu levels. This suggests that large yellow croaker could accumulate excess Cu in tissues. This phenomenon was also found in other species. Berntssen et al. (1999) indicated that the whole-body Cu concentration in Atlantic salmon increased with the increasing of dietary copper levels, especially when the dietary copper contents reached to 500 mg $kg^{-1}$ and 900 mg $kg^{-1}$. Lee and Shiau (2002) showed that with the increase of dietary copper levels (1.54–252.55 mg $kg^{-1}$), the whole-body Cu concentration in grass shrimp also increased. The vertebrae Cu concentration has the similar trends with the whole-body Cu concentrations in the present study. In general, after being fed with increasing levels of dietary copper for about 1 week or more, Cu would accumulate to the higher concentration in intestine and liver, and to the lower concentration in gill, muscle and kidney (Baker et al., 1998; Clearwater et al., 2000; Kamunde et al., 2001). Based on the whole-body and vertebrae Cu concentrations, the minimum requirements of dietary copper for the juvenile large yellow croaker were estimated to be 5.30 and 5.90 mg $kg^{-1}$ (Figs. 3 and 4), respectively. They were higher than the requirement based on the growth data (3.41 mg $kg^{-1}$). The same thing was also found in the research on Atlantic salmon (Lorentzen et al., 1998), in which 8.5–13.7 mg $kg^{-1}$ of dietary copper was determined as the minimum requirement based on the liver Cu concentration.

As an antioxidant enzyme, the superoxide dismutase (SOD) plays an important role in the protection of cells from free radical damages (Fang et al., 2002). The total antioxidant capacity (T-AOC) is one of the important indexes of the body's antioxidant effect, decompose and removal the reactive oxygen species (ROS). The Cu-Zn SOD has been shown to be a good indicator for the copper nutrition status in channel catfish (Gatlin and Wilson, 1986), flounder (Wei et al., 2001), grouper (Lin et al., 2008; Lin et al., 2010), abalone (Wang et al., 2009) and the Chinese mitten crab (Sun et al., 2013). Studies with channel catfish (Gatlin and Wilson, 1986) and abalone (Wang et al., 2009) suggested that the activity of Cu-Zn SOD was significantly decreased when fed diets with high copper levels. In the present study, the activity of Cu-Zn SOD followed a similar trend to those in channel catfish and abalone. Studies in blunt snout bream *Megalobrama amblycephala* (Shao et al., 2012) showed that the T-AOC in fish fed diets with increasing copper contents

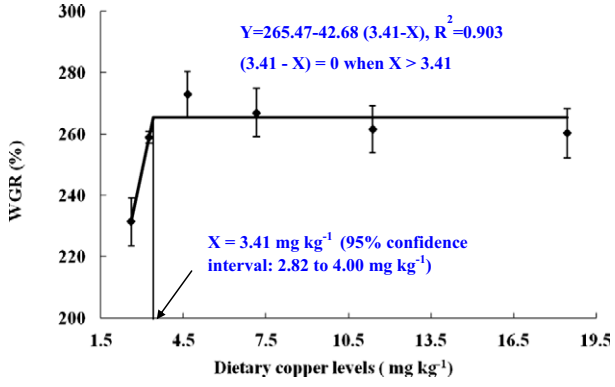


**Fig. 1.** Broken-line analysis of the relationship between dietary copper level and weight gain rate (WGR) indicates that the optimal dietary copper content was 3.41 mg $kg^{-1}$. Each point represents the mean of three replicates.

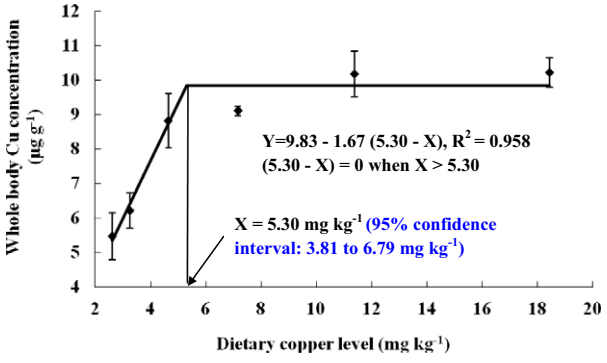


**Fig. 3.** Broken-line analysis of the relationship between dietary copper level and the whole-body Cu concentration indicates that the minimum dietary copper content was 5.30 mg $kg^{-1}$. Each point represents the mean of three replicates.

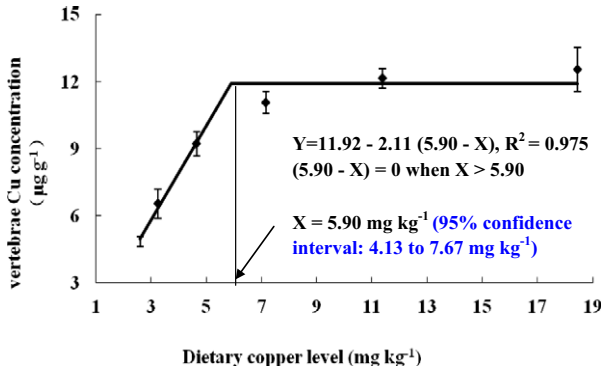


**Fig. 4.** Broken-line analysis of the relationship between dietary copper level and vertebrae Cu concentration indicates that the minimum dietary copper content was 5.90 mg $kg^{-1}$. Each point represents the mean of three replicates.

(from 9 to 100 mg $kg^{-1}$) was significantly higher than that fed the control (0 mg $kg^{-1}$) or the highest copper (150 mg $kg^{-1}$) diet ($P < 0.05$). The T-AOC and Cu-Zn SOD activity, in the present study, shared a same changing trend (Table 5). Both Cu-Zn SOD activity and the T-AOC in serum had the highest value when dietary copper content was 7.16 mg $kg^{-1}$. Meanwhile, when dietary copper content was 4.65 mg $kg^{-1}$, the Cu-Zn SOD activity and T-AOC in liver reached the highest. The abovementioned data showed that the dietary copper contents increasing within a certain range improved the anti-oxidative capability. However, excessive copper is detrimental to this capability. It is likely that the body was stimulated to produce compensatory response to improve the T-AOC at low dietary copper level. Then in higher copper, T-AOC decreased due to the Cu-Zn SOD activity was inhibited.

## 5. Conclusion

In conclusion, dietary copper is essential for the growth of large yellow croaker. Based on the WGR, the optimal dietary copper content for this species 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.

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