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The protective effects of dietary zinc on dietary copper toxicity in large yellow croaker *Larimichthys croceus*

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ABSTRACT

A study was carried out to test the responses of juvenile large yellow croaker to dietary copper and zinc intakes. Four experimental diets (coded as: −Cu − Zn, −Cu + Zn, +Cu − Zn and +Cu + Zn) were formulated containing two levels of Cu (4 and 24 mg/kg diet) and two levels of Zn (0 and 120 mg/kg diet), and each diet was fed to fish in triplicate for 10 weeks. Fish fed with +Cu diets (+Cu − Zn and +Cu + Zn) had lower final body weight, weight gain, superoxide dismutase, catalase, glutathione peroxidase (GPX), 6-phosphogluconate dehydrogenase, malic enzyme, isocitrate dehydrogenase and fatty acid synthase activities than fish fed with −Cu diets (−Cu − Zn and −Cu + Zn), but fish fed with +Cu diets had higher liver Cu, muscle Cu and thiobarbituric acid reactive substance (TBARS) contents, and lipoprotein lipase (LPL) activity than fish fed with −Cu diets. By contrast, fish fed with +Zn diets (+Cu + Zn and −Cu + Zn) had greater GPX activities than fish fed with −Zn diets (+Cu − Zn and −Cu − Zn), but fish fed with +Zn diets had lower TBARS content and LPL activity than fish fed with −Zn diets. This study indicated that high copper intake reduced growth of juvenile large yellow croaker, inhibited activities of antioxidant enzymes and lipid synthetases. The high levels of dietary zinc could mitigate the adverse effect of high copper toxicity on fish performance.

Statement of relevance: This study will be useful in developing mineral-balanced diets for intensive culture of large yellow croaker.

1. Introduction

Copper (Cu) is an essential trace element for vertebrates and is involved in numerous important biochemical reactions (Lall, 2002). Despite the essential role of Cu in a number of key enzymes, such as superoxide dismutase, tyrosinase and lysyl oxidase (Watanabe et al., 1997), it is deleterious at excessive concentrations (Lapointe et al., 2011). At present, the contamination of global ecosystems by metals is one of the main environmental issues, heavy metal of aquatic feed ingredients (e.g. fish meal and soybean meal) excess levels are difficult to control (Clearwater et al., 2002). Dietary Cu deficiency has been shown to reduce appetite, growth and anaemia in animals (Lin et al., 2008), however, thus far fundamental research on the dietary excessive copper on fish growth has been limited. Recently, Ye et al. (2013) reported that the pre-exposure to zinc (Zn) could reduce the harmful effect of Cu against fish. However, to our knowledge, the effect of the high Zn intake on the Cu toxicity in fish is not yet well studied.

Zn is also an essential trace metal and vital to various biological processes and functions, such as cell structure, hormone secretion, enzyme activities, protein and carbohydrate metabolism (Watanabe et al., 1997). In vertebrates, the accumulation of Zn goes along with increased abundance of metallothioneins (MTs), which are essential for the regulation of intracellular heavy metal homeostasis and detoxification (Sutherland and Stillman, 2011). Fosmire (1990) reported that a chronic intake of Zn can lead to severe Cu deficiency in humans, likely due to MT induction and Cu fixation in intestinal epithelial cells. Studies in rats suggested an additive effect of Zn and Cu on MT expression and protein abundance (Blalock et al., 1988). Thus, it is likely that feeding high dietary Zn levels with subsequent Zn accumulation and MT induction would also affect Cu elements.

Large yellow croaker *Larimichthys croceus* is a commercially important marine species, highly preferred by consumers, and widely cultured in China. To our knowledge, no information is available concerning the effect of dietary copper and zinc levels on growth performance, antioxidant status and lipid metabolism enzyme activity of juvenile large yellow croaker. The results could shed light to enhance fish growth and health through dietary formulation.

2. Materials and methods

2.1. Experimental diets and animals

Four experiment diets were formulated containing approximately 45.76% protein and 9.69% lipid, including two levels of Cu (4 and 24 mg/kg diet; Cao et al. (2014) reported that a dietary Cu requirement

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Table 1
Ingredient and chemical proximate composition of the experimental diets (% dry weight).

	−Cu − Zn	−Cu + Zn	+Cu − Zn	+Cu + Zn
Ingredients				
Casein, vitamin-free[a]	36.0	36.0	36.0	36.0
Gelatin	9.00	9.00	9.00	9.00
Dextrin	25.0	25.0	25.0	25.0
Fish oil	9.00	9.00	9.00	9.00
Lecithin	3.00	3.00	3.00	3.00
Vitamin premix[b]	2.00	2.00	2.00	2.00
Mineral premix, Cu, Zn-free[c]	1.00	1.00	1.00	1.00
Betaine	1.00	1.00	1.00	1.00
Monocalcium phosphate	1.00	1.00	1.00	1.00
Microcrystalline cellulose	12.9	12.9	12.9	12.9
Ethoxyquin	0.05	0.05	0.05	0.05
$CuSO_4 \cdot 5H_2O$ (mg/kg diet)	0.00	0.00	20.0	20.0
$ZnSO_4 \cdot 7H_2O$ (mg/kg diet)	0.00	100	0.00	100
Proximate composition				
Crude protein (%)	45.8	45.1	46.2	45.9
Crude lipid (%)	9.48	9.32	10.1	9.84
Copper (mg/kg)	3.78	4.82	24.3	26.1
Zinc (mg/kg)	22.1	125	21.9	124

[a] Casein, vitamin free: crude protein 93.01%, crude lipid 0.81% (Gansu Hualing Casein Co. Ltd., Gansu, China).
[b] Vitamin premix (mg/kg diet): retinol acetate, 32; cholecalciferol, 5; menadione sodium bisulfite, 5.1; α-tocopherol, 120; thiamin-HCl, 25; riboflavin, 36.7; pyridoxine-HCl, 20; vitamin B_{12}, 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.
[c] Mineral premix (mg/kg diet): $MgSO_4 \cdot 7H_2O$, 1826; $FeSO_4 \cdot 7H_2O$, 119; $MnSO_4 \cdot H_2O$, 44; $CoCl_2 \cdot 6H_2O$, 2; Na_2SeO_3, 0.45; $Ca(IO_3)_2 \cdot 6H_2O$, 2.35;cellulose, 7996.

in juvenile large yellow croaker was 3.41 mg/kg diet) and Zn (0 and 120 mg/kg diet; Zhang et al. (2008) reported a dietary Zn requirement in juvenile large yellow croaker was 59.6 mg/kg diet.). The four dietary combinations were coded as −Cu − Zn, Cu + Zn, +Cu − Zn and +Cu + Zn, respectively. Diets were processed into 3 mm diameter pellets, dried at room temperature to <10% moisture, ground and sieved to appropriate size before being stored at −20 °C. The formulation and proximate composition of each diet is presented in Table 1.

Juvenile large yellow croaker was obtained from a fish farm in Ningde (China). The fish were reared in floating sea cages (3.0 × 3.0 × 3.0 m) and were fed the commercial diet for 14 days. Fish (4.49 ± 0.29 g, mean ± S.E.M.) were randomly stocked into 12 sea cages (1.5 × 1.5 × 2.0 m) with 60 fish each in triplicate. The fish were hand-fed experimental diets twice (05:00–06:00 and 17:00–18:00) to apparent satiation for 10 weeks. During the trial, the water temperature ranged from 21.0 °C to 25.0 °C, salinity 22–26‰ and dissolved oxygen concentration was about 6.8 ± 0.15 mg/L.

2.2. Sampling

At the end of the experimental, fish were starved for 24 h, and then were anesthetized with tricaine methanesulfonate (MS–222) at 120 mg/L for weighing, counting and measurement. Five fish of each cage were minced and pooled, then stored at −20 °C for the analysis proximate composition of whole body. Three individuals of each per cage were weighed and livers were removed to determine the hepatosomatic index, and livers were stored at −80 °C for analysis of Cu concentration, antioxidant enzyme activity and fatty acid metabolism enzyme. Muscle (3 fish/cage) were sampled and stored at −20 °C for Cu concentration.

2.3. Biochemical composition analysis

All experimental diets and fish samples were analyzed in triplicate for proximate composition following the standard methods (AOAC, 2000). Moisture was determined by oven drying at 105 °C to a constant weight. The samples used for dry matter were digested with nitric acid and incinerated in a muffle furnace at 550 °C overnight for ash determination. Protein was measured by the combustion method using FP-528 nitrogen analyzer (Leco Corporation, St. Joseph, MI, USA). Lipid was determined by ether-extraction method using the Soxtec System HT (Foss FOSS Tecator HT6, Hoganos, Sweden). Cu and Zn concentrations were determined by the inductively coupled plasma-atomic emission spectrophotometer (Vista-MPX, Varian).

2.4. Antioxidant enzyme activity and lipid peroxidation assays

The frozen livers were weighed and homogenized in ice-cold phosphate buffer (50 mM, pH 7.4). The homogenate was centrifuged at 20,000*g* in a cooling centrifuge at 4 °C for 15 min and the supernatant was saved. Total superoxide dismutase (SOD) activity was determined following the methods of Beauchamp and Fridovich (1971). Catalase (CAT) activity was determined by measuring the decrease in H_2O_2 concentration (Aebi, 1984). Glutathione peroxidase (GPX) activity was measured following the methods of Flohé and Günzler (1984). Glutathione reductase (GR) activity was measured following the methods of Ching et al. (2009). The terminal product formed in the decomposition of polyunsaturated fatty acids mediated by free radicals was quantified as thiobarbituric acid reactive substances (TBARS) according to the methods of Buege and Aust (1978). All assays were determined with commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) in accordance with the manufacturer's instructions.

2.5. Lipid metabolism enzyme activity assays

The frozen liver were weighed and homogenized in ice-cold buffer (0.02 M Tris-HCl, 0.25 M sucrose, 2 mM EDTA, 0.1 M sodium fluoride, 0.5 mM phenyl methyl sulphonyl fluoride, 0.01 M β-mercapo-ethanol, pH 7.4). The homogenate was centrifuged at 20,000*g* in a cooling centrifuge at 4 °C for 30 min and the supernatant was saved. 6-phosphogluconate dehydrogenase (6PGD) and Glucose-6-phosphate dehydrogenase (G6PD) were determined by the method of Barroso et al. (1999), malic enzyme (ME) activity following Wise and Ball

Table 2
Survival and growth performance of juvenile large yellow croaker.

Treatment	−Cu		+Cu		*P* value		
	−Zn	+Zn	−Zn	+Zn	Cu	Zn	Cu × Zn
FBW (g)	23.2 ± 0.82^{B}	23.0 ± 0.85^{B}	21.2 ± 0.45^{A}	22.1 ± 1.43^{A}	0.03	0.52	0.31
WG (g)	18.8 ± 0.66^{B}	18.5 ± 1.27^{B}	16.7 ± 0.50^{A}	17.6 ± 1.19^{A}	0.03	0.54	0.32
FE	1.35 ± 0.02	1.30 ± 0.07	1.33 ± 0.06	1.31 ± 0.09	0.93	0.36	0.67
HSI (%)	1.31 ± 0.03	1.28 ± 0.04	1.34 ± 0.04	1.34 ± 0.06	0.12	0.58	0.58
CF	1.46 ± 0.04	1.47 ± 0.07	1.48 ± 0.03	1.51 ± 0.02	0.36	0.49	0.66
[a]Survival (%)	80.6 ± 4.19	85.0 ± 1.67	80.0 ± 4.41	80.6 ± 3.47	0.26	0.26	0.38

Data are means of triplicate. Different superscript letters ($^{A, B}$) indicate a significant effect of copper ($P < 0.05$). FBW: final body weight; Weight gain (WG, g) = final individual weight (g) − initial individual weight (g); Feed efficiency (FE) = wet weight gain (g) / dry diet fed (g); Hepatosomatic index (HSI, %) = 100 × liver mass (g) / body mass (g); Condition factor (CF) = 100 × weight gain (g) / body length (cm)^3; Survival (%) = 100 × (finial number of fish) / (initial number of fish).
[a] During the experiment, average survival rate of larger yellow croaker is about 60%, due to a major outbreak of white spot disease occurred in this sea area.