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Effect of high dietary copper on growth, antioxidant and lipid metabolism enzymes of juvenile larger yellow croaker *Larimichthys croceus*

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ABSTRACT

A study was carried out to test the responses of juvenile larger yellow croaker *Larimichthys croceus* to high Cu intake. Experimental diets were formulated containing three levels of Cu: low Cu (3.67 mg/kg), middle Cu (13.65 mg/kg) and high Cu (25.78 mg/kg), and each diet were fed to large yellow croaker in triplicate for 10 weeks. Final body weight, weight gain and feed intake were the lowest in high Cu group, but hepatosomatic index was the highest; Cu concentrations in the whole-body, muscle and liver of fish fed low Cu diet was the lowest; Liver superoxide dismutase, catalase and glutathione peroxidase activities in fish fed high Cu diet were lower than those in fish fed other diets; The higher content of liver thiobarbituric acid reactive substance content was found in high Cu group, followed by middle Cu group, and the lowest in low Cu group; Liver 6-phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase, malic enzyme, isocitrate dehydrogenase and fatty acid synthase activities were the lowest in high Cu group, but lipoprotein lipase activity was the highest. This study indicated that high copper intake reduced growth of juvenile larger yellow croaker, inhibited activities of antioxidant enzymes and lipid synthetases, and led to energy mobilization.

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1. Introduction

Copper (Cu) is an essential trace element for fish and involved in numerous important biochemical reactions, such as hematopoiesis (Shao et al., 2010) and collagen synthesis (Lall, 2002). It is also important as a part of antioxidant enzymes (e.g. Cu–Zn SOD) and involved in oxidation–reduction reaction (Watanabe et al., 1997). However, the excessive accumulation of copper in the body causes toxicity (Lapointe et al., 2011). To date, it has become a challenge to keep the quantities of heavy metals in aquatic animal feed ingredients (e.g. fish meal, soybean meal and flour) under proposed maximum levels while heavy metal pollution is one of the major environmental issues (Clearwater et al., 2002). The previous studies found that exposure to Cu might affect fish growth performance (Chen et al., 2012), development (Carreau and Pyle, 2005) and reproduction (Pickering et al., 1977). Recently, Chen et al. (2013) found that Cu exposure could influence fish lipid deposition and lipid metabolism, the activities of lipogenic enzymes (e.g. fatty acid synthase) as well as mRNA levels of related genes decreased with increasing Cu concentrations, but activity and mRNA level of lipoprotein lipase gene increased. This study provides evidence that Cu exposure can disturb the normal processes of lipid metabolism of fish at both the enzymatic and molecular levels. At present, these studies mentioned above were conducted to determine the toxicity of Cu exposure, however, effect of high dietary Cu on fish growth, physiological and lipid metabolism remains unknown.

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 high dietary Cu on growth performance, antioxidant and lipid metabolism enzymes activities on juvenile larger yellow croaker. The results could shed light to enhance fish growth and health through dietary formulation.

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

	Low Cu	Middle Cu	High Cu
Ingredients			
Vitamin free casein	36.00	36.00	36.00
Gelatin	9.00	9.00	9.00
Dextrin	25.00	25.00	25.00
Fish oil	9.00	9.00	9.00
Lecithin	3.00	3.00	3.00
Vitamin premix[a]	2.00	2.00	2.00
Cu-free mineral mix[b]	1.00	1.00	1.00
Betaine	1.00	1.00	1.00
$Ca(H_2PO_4)_2$	1.00	1.00	1.00
Cellulose	12.95	12.95	12.95
Ethoxyquin	0.05	0.05	0.05
$CuSO_4 \cdot 5H_2O$ (mg/kg diet)	0.00	8.00	20.00
Proximate composition			
Moisture (%)	9.05	9.60	9.12
Crude protein (%)	45.91	46.01	45.58
Crude lipid (%)	9.71	9.83	10.01
Cu (mg/kg diet)	3.67	13.65	25.78

[a] Vitamin mix (mg or g/kg diet): thiamin, 25 mg; riboflavin, 45 mg; pyridoxine·HCl, 20 mg; vitamin B_{12}, 0.1 mg; vitamin K_3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin acid, 200 mg; folic acid, 20 mg; biotin, 1.2 mg; retinol acetate, 32 mg; cholecalciferol, 5 mg; α-tocopherol, 120 mg; ascorbic acid, 2000 mg; choline chloride, 2500 mg; microcrystalline cellulose, 14.1617 g.

[b] Cu-free mineral mix (mg or g/kg diet): $MgSO_4 \cdot 7H_2O$, 1200 mg; $FeSO_4 \cdot H_2O$, 80 mg; $MnSO_4 \cdot H_2O$, 44 mg, $ZnSO_4 \cdot H_2O$, 50 mg; $CoCl_2 \cdot 6H_2O$, 50 mg; Na_2SeO_3, 20 mg; $Ca(IO_3)_2$, 60 mg; $Ca(H_2PO_4)_2 \cdot H_2O$, 3000 mg; microcrystalline cellulose, 15.485 g.

2. Materials and methods

2.1. Experimental diets and animals

Three experimental diets, using casein and gelatin as protein sources and fish oil as the lipid source, were formulated to contain three graded levels of Cu [low Cu (3.67 mg/kg diet), middle Cu (13.65 mg/kg diet) and high Cu (25.78 mg/kg diet); Cao et al. (2014) reported that a dietary Cu requirement in juvenile large yellow croaker was 3.41 mg/kg diet]. 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 (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. At the end of the acclimation, fish (5.27 ± 0.17 g, mean ± S.E.M.) were randomly stocked into 9 sea cages (1.5 × 1.5 × 2.0 m) with 60 fish each in triplicate. The fish were hand-fed experimental diets twice daily (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. Experimental designs and sampling

At the termination of trial, all 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 from each cage were randomly sampled, minced, pooled and stored at −20 °C for the analysis of whole-body proximate composition and Cu concentration. Muscle (3 fish/cage) were sampled and stored at −20 °C for Cu concentration. Livers (3 fish/cage) were removed and individually weighed to determine the hepatosomatic index, and stored at −20 °C for Cu concentration, antioxidant and fatty acid metabolism enzymes activities analysis.

2.3. Biochemical composition analysis

All experimental diets and tissue samples were analyzed for proximate composition following the standard methods in triplicate (AOAC, 1995). 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. The protein was measured by the combustion method using an FP-528 nitrogen analyzer (Leco Corporation, St. Joseph, MI, USA). Lipid was determined by ether-extraction method using the Soxtec System HT (FOSS Tecator HT6, Hoganos, Sweden). Cu concentrations in the whole body and liver were determined by the inductively coupled plasma-atomic emission spectrophotometer (Vista-MPX, Varian).

2.4. Antioxidant enzyme activity and lipid peroxidation assays

The levels of enzyme activity and lipid peroxidation were measured with commercial assay kits (Nanjing Jiancheng Institute, Nanjing, China) in accordance with the instructions of the manufacturer. The assays are briefly described as follows:

The frozen liver were weighed and homogenized in ice-cold phosphate buffer (50 mM, pH 7.4). The homogenate was centrifuged at 2000 × *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). The ratio of auto-oxidation rates of the samples with or without homogenate was determined at 550 nm. One unit of SOD activity was calculated using the amount of superoxide dismutase required to inhibit the reduction of nitrobluete trazolium by 50%. Catalase (CAT) activity was determined by measuring the decrease in H_2O_2 concentration (Aebi, 1984). After 10 μL of homogenate was added to the reagent, the sample was incubated for 60 s at 37 °C. The absorbance of the samples was read at 405 nm. One unit of CAT activity was defined as the amount of CAT required to transform 1 μmol of H_2O_2 per min. Glutathione peroxidase (GPX) activity was measured following the methods of Flohé and Günzler (1984). After the addition of 1 mmol GSH (reduced glutathione) the NADPH-consumption rate was monitored at 412 nm. One unit of GPX activity was definedas the amount of GPX required to oxidize 1 μmol of NADPH per min. 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). Soluble protein concentration of homogenates was determined by the method of Bradford (1976) using bovine serum albumin (BSA) as standard.

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 homogenatewas 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 (1964), isocitrate dehydrogenase (ICDH) activity according to Bernt and Bergmeyer (1974), fatty acid synthase (FAS) activity according to the method of Chakrabarty and Leveille (1969). One unit of enzyme activity was defined as the amount of enzyme that converted 1 μmol of substrate to product per min at 30 °C. Lipoprotein lipase (LPL) activity was measured according to the modified method of Ballart et al. (2003). One unit of LPL activity was defined as the amount of enzyme that catalyzed

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