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Effects of dietary copper on survival, growth and immune response of juvenile abalone, *Haliotis discus hannai* Ino

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ABSTRACT

A study was conducted to evaluate the effects of dietary copper (Cu) on the growth, survival, carcass composition and immune responses in juvenile abalone, *Haliotis discus hannai*. Six semi-purified diets containing graded levels of dietary copper (1.08, 3.76, 6.54, 14.80, 26.84 and 109.41 mg/kg diet) from $CuSO_4 \cdot 5H_2O$ were fed to juvenile abalone (initial shell length: 17.21 ± 0.04 mm; initial body weight: 0.65 ± 0.00 g) in triplicate groups for 24 weeks in a flow-through system. The results showed that no significant ($P > 0.05$) differences were found in weight gain rate (WGR, %), daily increment in shell length (DISL, μm/day) and survival among the dietary treatments. However, Cu concentrations in abalone serum, hepatopancreas, muscle and newly grown shell showed a clear increasing trend with the supplementation of dietary Cu, and the concentrations were significantly ($P < 0.05$) higher in abalone fed dietary Cu ≥ 26.84 mg/kg compared to those fed 1.08 mg Cu/kg. The crude lipid in abalone carcass decreased with increasing dietary Cu and was significantly ($P < 0.05$) lower in abalone fed dietary Cu ≥ 26.84 mg/kg compared to the rest of the treatments. The activities of copper–zinc superoxide dismutase (CuZn SOD) in the hepatopancreas and serum were significantly increased when supplementation of dietary Cu reached 3.76 mg/kg diet, and 6.54 mg/kg diet provided maximum activity of serum phenoloxidase (PO). There was no significant difference for these enzyme activities when dietary Cu was over those values. The optimum requirement of juvenile abalone for dietary Cu was estimated to be 3–5 mg/kg diet by broken-line regression analysis, based on the activities of CuZn SOD and PO either in abalone hepatopancreas or serum. Deficiency of dietary Cu significantly depressed the immune responses in abalone.

1. Introduction

Copper (Cu) is an essential trace element for humans and animals for a number of biochemical functions (Davis and Mertz, 1987; Lall, 2002). The quantitative requirement for dietary copper has been reported for a few fish species including rainbow trout and carp (Ogino and Yang, 1980), channel catfish (Gatlin and Wilson, 1986), Atlantic salmon (Lall and Hines, 1987; Lorentzen et al., 1998), tilapia (Shiau and Ning, 2003) and grouper (Lin et al., 2008). Copper deficiency suppressed the growth of carp (Ogino and Yang, 1980) and reduced the activities of heart cytochrome c oxidase and liver copper–zinc superoxide dismutase (CuZn SOD) in channel catfish (Gatlin and Wilson, 1986). However, Cu is not only an essential but also a potentially toxic trace element, depending on its concentration. High level of dietary Cu can result in toxicity which has been confirmed in channel catfish (Murai et al., 1981) and rainbow trout (Lanno et al., 1985). Reduced growth and feed conversion efficiency (FCE) were observed in channel catfish fed 16 and 32 mg Cu/kg diet, and significantly higher level of liver Cu was deposited in fish fed 32 mg Cu/kg diet than those fed the control diet containing 1.5 mg Cu/kg diet (Murai et al., 1981). Dietary Cu toxicity occurred in rainbow trout at 730 mg Cu/kg diet, and was characterized by reduced growth and FCE, food refusal and elevated liver copper level (Lanno et al., 1985).

Abalone, *Haliotis discus hannai*, is a macro-algivorous marine gastropod, and one of the most commercially important species in aquaculture. There is no information on the requirement for dietary Cu in any species of mollusks so far, including abalone. Also, limited information is available on the nutritional immunology of this species (Chen et al., 2005). Hence, a one-factorial experiment was designed to evaluate the effects of dietary copper on the growth, survival, carcass compositions and immune responses in juvenile abalone, and to determine the optimum dietary copper requirement for this abalone species.

2. Materials and methods

2.1. Experimental diets and design

The basal diet was formulated (Table 1) to provide 30% crude protein from casein and gelatin and 3.5% crude lipid from soybean oil and menhaden fish oil (1:1), which were sufficient to support optimal

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Table 1
Ingredients and proximate composition of the basal diet.

Ingredients	Percents in diet (%, dry weight)
Casein	25.00
Gelatin	6.00
Dextrin	33.50
CM-cellulose	5.00
Sodium alginate	20.00
Vitamin mix[a]	2.00
Cu-free mineral mix[b]	4.50
Choline chloride	0.50
SO/MFO[c]	3.50
Proximate analysis ($n=3$)	
Crude protein (%)	30.54
Crude lipid (%)	3.72
Ash (%)	11.82
Copper (mg/kg)	1.08

[a] Vitamin mix, each 1000 g of diet contained: thiamin HCl, 120 mg; riboflavin, 100 mg; folic acid, 30 mg; pyridoxine HCl, 40 mg; niacin, 800 mg; Ca pantothenate, 200 mg; inositol, 4000 mg; biotin, 12 mg; vitamin B_{12}, 0.18 mg; ascorbic acid, 4000 mg; vitamin E, 450 mg; menadione, 80 mg; retinal acetate, 100,000 IU; cholecalciferol, 2000 IU.
[b] Cu-free mineral mix, each 1000 g of diet contained: NaCl, 0.4 g; $MgSO_4 \cdot 7H_2O$, 6.0 g; $NaH_2PO_4 \cdot 2H_2O$, 10.0 g; KH_2PO_4, 12.8 g; $Ca(H_2PO_4)_2 \cdot H_2O$, 8.0 g; Fe-citrate, 1.0 g; $ZnSO_4 \cdot 7H_2O$, 141.2 mg; $MnSO_4 \cdot H_2O$, 64.8 mg; $CoCl_2 \cdot 6H_2O$, 0.4 mg; KIO_3, 1.2 mg; Na_2SeO_3, 0.4 mg.
[c] Soybean oil : menhaden fish oil = 1:1.

growth (Mai et al., 1995a,b). The compositions of vitamin and mineral mixtures were modified from those used by Uki et al. (1985) without copper supplementation.

The graded levels of dietary Cu were designed according to dietary copper requirements (3–6 mg Cu/kg diet) of finfish (Ogino and Yang, 1980; Gatlin and Wilson, 1986; Lall and Hines, 1987; Lorentzen et al., 1998; Shiau and Ning, 2003; Lin et al., 2008). The diet without copper supplementation was considered the Cu-deficient group (named as basal diet). The high dose group was set at 120 mg Cu/kg according to Murai et al. (1981) who suggested that the toxic-dose level for channel catfish was about 20 times higher than the optimal dietary Cu level. Hence, the basal diet was supplemented with 0, 3, 6, 15, 30 and 120 mg Cu/kg diet from $CuSO_4 \cdot 5H_2O$ (Analytical Reagent, Shanghai Chemical Co., Shanghai, China), and the corresponding measured Cu concentrations in the diets were: 1.08, 3.76, 6.54, 14.80, 26.84 and 109.41 mg Cu/kg diet, respectively, as determined by inductively coupled plasma-atomic emission spectrophotometer (ICP-OES; VISTA-MPX, VARIAN) (Tan and Mai, 2001). Procedures for diet preparation were similar to those described by Mai et al. (1995a,b). The dietary flakes were sealed in sample bags and stored at −20 °C until use.

2.2. Animal rearing

Abalone juveniles were obtained from a spawning in October 2006 at Laoshan Fisheries Co., Shandong, China. Prior to initiation of the experiment, abalone were acclimated to laboratory conditions and fed the basal diet (Table 1) for 2 weeks. After measuring and recording the shell length and body weight, abalone (initial shell length: 17.21 ± 0.04 mm; initial body weight: 0.65 ± 0.00 g) were assigned to a flow-through system using a completely randomized design with six treatments and triplicate groups for each treatment. Each replicate was stocked with 45 abalone in a 100-l fiberglass tank containing a black waved board as a shelter. Each diet was fed to satiation to abalone once daily (17:00) for 24 weeks. Every morning, feces and excess diet were cleaned to maintain water quality. During the experimental period, the water temperature ranged from 10 to 20 °C, salinity from 28 to 31, pH from 7.8 to 8.1, and dissolved oxygen was ≥7.65 mg/l. The copper and zinc concentration was not detectable in the rearing water which was provided by natural seawater, and iron concentration was less than 0.001 mg/l.

2.3. Leaching

Abalone is a slow feeder. The fact found by Mai et al. (1998) is that the digestive tracts of most abalone are full of food within 2 h after feeding with the premium quality diets. In order to determine the leaching of dietary Cu during feeding, a leaching test was carried out using the method described by Tan and Mai (2001). Pre-weighed diet (10 g) was placed onto 100-μm-mesh screen and allowed to settle to the bottom of experimental system without abalone. Temperature, aeration and water flow rate were adjusted to match those of the feeding trial. At the end of allotted time (0, 2 and 6 h, respectively), the remained diet was removed from the system and dried overnight at 60 °C in an oven. Dried diet was submitted for analysis of total Cu by ICP-OES.

2.4. Sample collection

At the end of the feeding trial, animals were fasted for 3 days to empty the digestive system. All abalone were counted, weighed and shell lengths measured. Eight individuals from each replicate were sampled randomly for blood collection by cutting the blood sinus in the adductor muscle with a scalpel. Then the blood samples were centrifuged immediately at 3000 × *g* for 10 min at 4 °C to collect serum and stored at −70 °C for subsequent analyses.

After blood collection, hepatopancreas and muscle were removed from the same abalone, and stored at −70 °C for element and enzyme analyses. The methods of cleaning and drying the shell samples followed those described by Mai et al. (2003). Each shell obviously consisted of two parts, the brown old shell (OS) and the green newly grown shell (NS) formed after feeding the experimental diets. The NS was separated from OS, and both were prepared for the analyses of elements.

2.5. Sample analysis

Growth was expressed as the weight gain ratio (WGR, %) and daily increment in shell length (DISL, μm/day). The calculation formulae are as follows:

$$WGR = [(W_t - W_i) / W_i] \times 100$$

$$DISL = [(SL_t - SL_i) / t] \times 1000$$

where W_t and W_i are the final and initial mean weight (g), SL_t and SL_i are the final and initial mean shell length (mm), respectively, and t is the duration of the experiment in days.

The Cu concentration analyses in serum, hepatopancreas, muscle, OS, NS and experimental diets were conducted using the method described by Tan and Mai (2001). The serum and dried ground samples were digested in perchloric acid (70% $HClO_4$, ACS reagent at a ratio of 1:20 (w/v)). Then, the digested solution was appropriately diluted with Milli-Q water within the detectable range of ICP-OES (VISTA-MPX, VARIAN). Elemental concentrations of the samples were expressed on a dry-weight basis.

Proximate analyses to determine water content, protein, lipid, and ash in diets and animal tissues were conducted using standard procedures (Association of Official Analytical Chemists, AOAC, 1995). The protein concentration in the serum and hepatopancreas was spectrophotometrically measured according to the method of Bradford (1976) using bovine serum albumin as a standard. CuZn SOD activity in the hepatopancreas and serum was measured according to Gatlin and Wilson (1986). The activities of phenoloxidase (PO) and lysozyme in serum were measured using the method of Chen et al. (2005).