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Dietary zinc requirements of juvenile grouper, *Epinephelus malabaricus*

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ABSTRACT

Optimal dietary requirements of the juvenile grouper *Epinephelus malabaricus* for zinc (Zn) were investigated in an 18-week feeding trial, in which immune responses of the fish to the zinc treatments were also evaluated. In the feeding trial, the basal diet with egg white powder and mackerel muscle meal as the protein sources had a crude protein level of 52% and a residual zinc concentration of 10.0 mg/kg diet. Zn of graded levels (0, 4, 8, 12, 24, 36 and 140 mg Zn/kg diet as zinc sulfate) was added to the basal diet and fed to experimental fish with an initial mean weight of 5.9 g. The results showed that dietary zinc significantly affected the growth of the fish, but not the survival. The non-supplemental group showed a weight gain significantly lower than that of the supplemental groups. Zinc concentrations in fish serum, muscle, vertebrate and scales were increased with increasing Zn levels in diets. The dietary treatments did not significantly affect nonspecific immunity parameters including macrophage phagocytosis, alternative complement pathway activity (ACH50), agglutination titer and lysozyme activity as well as erythrocyte superoxide dismutase activity. Broken-line analyses based on the weight gain and body tissue zinc concentrations indicate that the optimal levels of dietary zinc for the juvenile grouper ranged between 28.9 and 33.7 mg/kg diet.

1. Introduction

Groupers are an economically important aquaculture and food fish in the Asia-Pacific region because of their desirable taste, rapid growth, efficient feed conversion and high market value (Millamena, 2002). The wholesale price of live market-size groupers was about USD 13/kg in Hong Kong and USD 16/kg in Bahrain (FAO, Food Agriculture Organization, 2005). Global grouper production increased dramatically from 60,774 mt in 1990 to 198,690 mt in 2007 (Harikrishnan et al., 2010). Among the groupers that are cultured, *Epinephelus malabaricus* is one of the most important species (Tucker, 1999). Compounded feeds are widely used in grouper aquaculture as the nutritional requirements of the fish have been extensively studied (Chen, 1998; Lupatsch and Kissil, 2005; Lupatsch et al., 2003). Among the essential nutrients, the requirements for many minerals have been reported, including calcium and phosphorus (9.3 and 10.3 g/kg diet, respectively; Ye et al., 2006), iron (100 mg/kg diet; Ye et al., 2007), copper (4–6 mg/kg diet; Lin et al., 2008) and selenium (0.6–0.8 mg/kg diet; Lin and Shiau, 2005b). The requirements for Zn, however, have not been reported.

Zinc serves essential structural, catalytic and regulatory functions in many biological systems (Eide, 2006; Maret and KrEl, 2007). Zn is an integral part of metalloenzymes, such as carbonic anhydrase and alkaline phosphatase, which warrant its roles in regulating many processes of carbohydrate, lipid and protein metabolisms. While fish obtain Zn from both water and diets, dietary source is more efficiently absorbed (Handy, 1996). The requirements of dietary Zn for many fishes, mostly freshwater fishes, have been reported including rainbow trout (Ogino and Yang, 1978), catfish (Gatlin and Wilson, 1983, 1984), red drum (Gatlin et al., 1991), Nile tilapia (Eid and Ghonim, 1994), hybrid tilapia (Lin, 2008), hybrid striped bass (Buentello et al., 2009) and grass carp (Liang et al., 2012). The requirements fall within the range between 15 and 70 mg/kg feed. Zn requirements of marine finfishes, in contrast, are less known. Ma et al. (2014) reported an increased bioavailability when turbots (*Scophthalmus maximus*) were fed chelated Zn.

Zn deficiency in fish has been demonstrated. In channel catfish, diets low in Zn cause reduced growth, appetite, bone Zn and calcium levels, and serum Zn concentrations (Gatlin and Wilson, 1983). Although Zn deficiency has been associated with immunological symptoms in mammals, such as thymus and spleen atrophy, and reduces macrophage and T cell activities and number of precursor cells (Sahin et al., 2005), relatively little is known of the relationship between dietary Zn and fish immunity. Channel catfish fed a zinc-free diet suffered total mortality when challenged with pathogenic *Edwardsiella ictaluri* (Paripatananont and Lovell, 1995b). Scarpa and Gatlin (1992) could not establish the link in channel catfish between Zn status and serum IgM, neutrophil no. and susceptibility to *Aeromonas hydrophila* challenge. Similarly, Lim et al. (1996) were unable to find a significant relationship between dietary Zn and abundance of white blood cells, macrophages and neutrophils in channel catfish.

In the present study, in addition to quantifying dietary Zn requirement of the juvenile groupers, we evaluated the effects of Zn supplement on non-specific immunity of the fish at the end of the feeding

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trial. The results are important as a basic knowledge in formulating cost-effective feeds for the groupers.

2. Materials and methods

2.1. Experimental diets and diet preparation

Seven experimental diets supplemented with graded levels of Zn in the form of $ZnSO_4$ were evaluated. The diets were formulated based on a basal diet (Table 1). Mackerel muscle meal (containing 17.6 mg Zn/kg) and egg white powder (Zn concentration not detectable) were used as the protein source (Gatlin et al., 1991). $ZnSO_4 \cdot 7H_2O$ (Nakarai Chemicals, Kyoto, Japan) was added at 0, 4, 8, 12, 24, 36 and 140 mg Zn/kg diet. The experimental diets were of the same ingredient composition except that a portion of cellulose was added to achieve Zn concentration gradation. The total Zn concentrations of the experimental diets were measured in duplicate using a flame atomic absorption analyzer (Z8200, Hitachi, Tokyo, Japan) to be 10.0, 17.6, 23.6, 26.4, 36.7, 47.3 and 145.5 mg/kg diet, respectively (Table 1), indicating that there was a residual Zn level of 10 mg/kg in the basal diet. The ingredients were mixed thoroughly and water was added to form a dough, and then passed through a mincer with a die of 4 mm in diameter. The resulting strands were air-dried at 20 °C. After drying, the diets were broken up and sieved. Pellets of 4 mm in diameter were collected and stored at −20 °C until used.

2.2. Experimental procedure

The experiment was conducted in accordance with the guideline of the University regarding research on experimental animals. Juvenile *E. malabaricus* with a mean body length of 7 cm were obtained from a commercial hatchery in southern Taiwan. The fish were raised indoors and acclimated to a commercial compounded feed for 1 month and then to the non-Zn-supplemental basal diet (Table 1) for 12 days when they reached an average body weight of 5.9 ± 1.7 g. Twelve apparently healthy fish were selected and assigned randomly to one of the 21 aquarium tanks, each with its own independent recirculating system. The water volume in each tank was 290 l and a volume of 16 l/min was recirculated. A part of the recirculated water (10–20%) was replaced daily with fresh seawater. The water temperature and salinity were controlled and maintained at 30 °C and at 29 ppt, respectively. Dissolved oxygen concentrations were monitored and were >5 mg/l during the feeding trial. Artificial illumination was provided to maintain a 12 h light/12 h dark cycle. The groupers were hand-fed twice daily with the experimental diets at a rate of 4% body weight per day. The feeding trial lasted for 18 weeks.

At the end of the feeding trial, the groupers were individually weighed. Body weight gain [100 × (final body weight − initial body weight) / initial body weight], feed efficiency [(final body weight − initial body weight) / feed intake], and survival [100 × (final fish number / initial fish number)] in each tank were calculated. Apart from the two largest fish reserved for the immunological study, five fish from each tank were randomly taken. Blood was sampled. Muscle, liver, vertebrate and scale were collected. Zn concentrations in these tissues were analyzed by the atomic absorption analyzer. The detection limit for Zn was 0.02 μg/ml.

For the immunological study, the two largest fish from each tank were injected intra-peritoneally with 10 mg beta-1,3-glucan (from *Schizophyllum commune*) per 100 g wet weight. The fish were returned to the same aquarium tank and fed continuously with the assigned experimental diets. Seven days after the injection, the fish were anesthetized and peritoneal exudate cells were collected. Lymphocytes were then harvested following centrifugation with Percoll. The macrophages were used to quantify phagocytic index (Matthews et al., 1990), alternative complement activity (ACH50, Sunyer and Tort, 1995; Tort et al., 1996), hemagglutination activity (Tort et al., 1996), lysozyme activity (Ellis, 1990) and superoxide dismutase activity (based on RANSOD kit analyses). The details of the experimental procedures were described previously (Wu and Chen, 2012).

2.3. Statistical analyses

The present study followed a completely randomized design with triplication in each treatment. Each measured response was analyzed by one-way analysis of variance (ANOVA) using SAS/PC software (SAS Inst. Inc., Cary, NC), and significance was set at $P < 0.05$. Multiple comparisons among means were performed with Duncan's new multiple range tests. Dietary Zn requirements of the grouper were estimated by the broken-line regression method (Robbins, 1986) based on weight

Table 1
Ingredient and chemical compositions of the experimental diets.

	Total zinc mg/kg diet						
	10.0	17.6	23.6	26.4	36.7	47.3	145.5
Ingredients							
Mackerel muscle meal[a]	374	374	374	374	374	374	374
Spray-dried egg white[b]	200	200	200	200	200	200	200
α-Starch	110	110	110	110	110	110	110
Corn starch	103	103	103	103	103	103	103
Fish oil	30	30	30	30	30	30	30
Soybean oil	10	10	10	10	10	10	10
Zinc-free mineral premix[c]	80	80	80	80	80	80	80
Vitamin premix[d]	40	40	40	40	40	40	40
Attractant[e]	17	17	17	17	17	17	17
Ca-lactate	35	35	35	35	35	35	35
Biotin	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Cellulose	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Chemical composition							
Crude protein (g/100 g)	52.5 ± 1.1	52.2 ± 0.5	51.7 ± 0.3	51.7 ± 0.4	51.9 ± 0.4	52.1 ± 0.4	51.8 ± 0.3
Crude lipid (g/100 g)	7.0 ± 0.3	6.0 ± 0.2	6.8 ± 0.3	6.4 ± 0.1	6.3 ± 0.1	6.5 ± 0.8	6.8 ± 0.1
Ash (g/100 g)	7.0 ± 0.1	6.9 ± 0.1	6.9 ± 0.1	6.9 ± 0.1	6.9 ± 0.1	6.9 ± 0.1	6.9 ± 0.1
Moisture (g/100 g)	8.4 ± 0.2	8.3 ± 0.2	7.6 ± 0.3	8.0 ± 0.2	7.5 ± 0.1	7.4 ± 0.1	9.6 ± 0.1
Zn concentration (mg/kg)	10.0 ± 2.4	17.6 ± 2.7	23.6 ± 3.3	26.4 ± 1.6	36.7 ± 3.9	47.3 ± 2.4	145.5 ± 9.6

[a] Lyophilized muscle of adult mackerel (*Scomberomorus commerson*), containing 17.6 mg Zn/kg dry weight.
[b] Containing undetectable level of zinc (<0.01 mg/kg).
[c] Following Sakamoto and Yone (1978).
[d] Following Wu and Chen (2012).
[e] Containing taurine, betaine, alanine and inosine-5′ monophosphoric acid at a weight-basis ratio of 6:6:4:1 (Kanazawa, 1997).