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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).

gain, serum Zn concentration, vertebra Zn concentration, and scale Zn concentration.

## 3. Results

Dietary Zn concentrations significantly affected weight gain and feed efficiency, but not survival (Table 2). Weight gain was highest ($P < 0.05$) in fish fed the diets with ≥36.7 mg Zn/kg, followed by fish fed the diet with 17.6–26.4 mg Zn/kg, and lowest in fish fed the diet with 10.0 mg Zn/kg (Table 2). Feed efficiency followed essentially the same pattern of weight gain. Fish mortality during the feeding trial was in average less than 3% (Table 2).

Zn concentrations in two soft tissues (Table 3) and two hard tissues (Table 4) were significantly affected by dietary Zn levels. Among the 3 soft tissues investigated, serum and muscle Zn concentrations were found to be significantly affected by the dietary treatments, but not the Zn concentration in the liver (Table 3). Zn concentrations in the two hard tissues were increased significantly with increasing dietary Zn levels (Table 4) and reached a plateau when the total Zn level in the diets was greater than 23.6 or 26.4 mg Zn/kg. Serum Zn concentration was significantly higher when fish were fed diets with ≥23.6 mg Zn/kg than diets with ≤17.6 mg Zn/kg. Muscle Zn concentration was highest in fish fed the diet with 145.5 mg Zn/kg. All of the immunological indices evaluated including phagocytic index, alternative complement activity, hemagglutination assay, lysozyme activity, and superoxide dismutase activity were not affected significantly ($P > 0.05$) by the treatments (Table 5).

The broken-line regression analyses of weight gain, serum Zn concentrations, vertebra Zn concentration and scale Zn concentration revealed respective broken points at 33.7, 32.7, 28.9 and 32.6 mg Zn/kg, respectively (Fig. 1), indicating that the adequate requirements of dietary Zn for the grouper ranged between 28.9 and 33.7 mg Zn/kg.

## 4. Discussion

Diets supplemented with Zn in the present study supported satisfactory growth of the groupers. The experimental fish grew more than 5 times in body weight during the 18-week period. Weight gain improved significantly with increasing dietary Zn provision and leveled off approximately at the total dietary level of 36.7 mg/kg (Table 2). The broken-line analyses based on weight gain and tissue Zn levels revealed a requirement ranging between 28.9 and 33.7 mg Zn/kg (Fig. 1). The known dietary Zn requirements varied among fishes such as channel catfish (20 mg/kg diet, Gatlin and Wilson, 1983; Paripatananont and Lovell, 1995a), red drum (20 mg/kg diet, Gatlin et al., 1991), Atlantic salmon (37–57 mg/kg diet, Maage and Julshamn, 1993), yellow catfish (17–21 mg/kg diet, Luo et al., 2010) and grass carp (55 mg/kg diet, Liang et al., 2012), as requirements of dietary minerals for aquaculture animals usually differ from each other due to the different parameters involved. In the present study, the lowest dietary Zn level was 10.0 mg/kg, which was achieved when no Zn was supplemented and most of Zn was from the mackerel muscle meal. The diet with the lowest Zn level rendered the lowest weight gain, feed efficiency and Zn concentration in most soft and hard tissues. Zn concentrations in muscle varied among groups, with the fish fed the diet with 145.5 mg Zn/kg having the highest muscle Zn concentration.

In *E. malabaricus*, Zn concentration in both vertebrate and scales became saturated when dietary Zn level was higher than 23.6 or 26.4 mg Zn/kg (Table 4). Serum Zn concentration became saturated when the dietary Zn level was higher than 23.6 mg Zn/kg (Table 3). In contrast, muscle Zn concentration was increased with increasing dietary provision, showing no saturation. Liver Zn concentration did not respond significantly to dietary treatments. Similar responses have also been reported for whole body Zn of grass carp (Liang et al., 2012), scale and bone Zn of blue tilapia and red drum (McClain and Gatlin, 1988; Gatlin et al., 1991), and whole body and plasma Zn of Nile tilapia (Eid and Ghonim, 1994). Our results on muscle responses are the first report in the effects of dietary zinc level on fish muscle Zn concentration.

The juvenile groupers fed the non-supplemental diet did not show apparent Zn-deficiency symptom. Many pathological symptoms including cataract, poor growth, high mortality, dermatitis, short trunk, low tissue Zn concentration and anorexia have been reported to associate with Zn deficiency in various fish species (Eid and Ghonim, 1994; Gatlin and Wilson, 1983; Ketola, 1979; Ogino and Yang, 1978; Satoh et al., 1987a, 1987b; Yamamoto et al., 1983). In the present study, no such symptoms were observed during the course of the 18-week trial. The unsupplemental experimental diet still contained a residual Zn concentration of 10.0 mg/kg, mainly from the fish muscle meal. It is likely that in order to succumb to deficiency, the grouper would require a diet with a Zn concentration lower than the 10 mg/kg level. Ogino and Yang (1979) found that when dietary Zn levels were higher than 5 mg/kg, deficiency symptoms in carp such as cataract and fin rod disappeared instantly. An extremely low dietary Zn level seems to be needed in order to cause Zn deficiency in many fishes, including the grouper.

Dietary Zn concentration did not affect any of the immunological indicators evaluated in the present study. These indicators covered a wide range of the indices commonly used to gauze immune capacity of

**Table 2**
Weight gain, feed efficiency and survival of juvenile grouper fed for 18 weeks with diets containing different levels of zinc[1].

| Total zinc (mg/kg) | Weight gain[2] (%) | Feed efficiency[3] | Survival (%) |
|---|---|---|---|
| 10.0 | 269.5 ± 56.3[a] | 0.50 ± 0.05[a] | 97.2 ± 4.8 |
| 17.6 | 419.7 ± 118.3[b] | 0.56 ± 0.10[ab] | 97.2 ± 4.8 |
| 23.6 | 483.7 ± 69.8[bc] | 0.61 ± 0.02[bc] | 100.0 ± 0 |
| 26.4 | 463.9 ± 31.3[bc] | 0.61 ± 0.04[bc] | 100.0 ± 0 |
| 36.7 | 587.2 ± 75.2[c] | 0.68 ± 0.03[c] | 97.2 ± 4.8 |
| 47.3 | 585.3 ± 55.4[c] | 0.66 ± 0.04[c] | 97.2 ± 4.8 |
| 145.5 | 555.3 ± 59.3[c] | 0.65 ± 0.05[bc] | 97.2 ± 4.8 |

[1] Means ± SD (n = 3). Means in each column with different superscript letters are significantly different ($P < 0.05$).
[2] WG (%) = 100 × (final body weight − initial body weight) / (initial body weight). Initial body weight of fish was 5.86 ± 1.73 g.
[3] Feed efficiency = wet weight gain (g) / dry feed intake (g).

**Table 3**
Zinc concentrations[1] (μg/g) in serum, muscle, and liver of juvenile grouper fed for 18 weeks with diets containing different levels of zinc.

| Total zinc (mg/kg) | Serum | Muscle | Liver |
|---|---|---|---|
| 10.0 | 3.4 ± 0.4[a] | 3.5 ± 0.5[a] | 21.3 ± 2.3 |
| 17.6 | 6.2 ± 0.4[a] | 4.4 ± 1.2[a] | 18.9 ± 3.0 |
| 23.6 | 14.2 ± 0.9[b] | 5.2 ± 0.2[ab] | 24.0 ± 1.7 |
| 26.4 | 14.4 ± 1.3[b] | 3.3 ± 0.2[a] | 20.9 ± 4.5 |
| 36.7 | 20.2 ± 1.9[b] | 3.5 ± 0.9[a] | 25.2 ± 3.5 |
| 47.3 | 18.0 ± 4.8[b] | 5.3 ± 1.4[ab] | 31.2 ± 5.1 |
| 145.5 | 18.3 ± 8.3[b] | 6.4 ± 1.7[b] | 26.0 ± 11.1 |

[1] Means ± SD (n = 3). Means in each column with different superscript letters are significantly different ($P < 0.05$).

**Table 4**
Zinc concentrations[1] (μg/g) in the hard tissues of juvenile grouper fed for 18 weeks with diets containing different levels of zinc.

| Total zinc (mg/kg) | Vertebra | Scale |
|---|---|---|
| 10.0 | 87.0 ± 14.4[a] | 131.4 ± 2.9[a] |
| 17.6 | 121.1 ± 13.7[b] | 219.5 ± 43.2[b] |
| 23.6 | 185.6 ± 12.0[c] | 335.2 ± 54.9[c] |
| 26.4 | 242.8 ± 13.1[d] | 272.8 ± 47.1[b] |
| 36.7 | 240.0 ± 0.3[d] | 395.0 ± 25.5[c] |
| 47.3 | 253.7 ± 13.9[d] | 366.5 ± 12.0[c] |
| 145.5 | 253.3 ± 9.6[d] | 391.0 ± 18.1[c] |

[1] Means ± SD (n = 3). Means in each column with different superscript letters are significantly different ($P < 0.05$).

**Table 5**
Phagocytic index of macrophages, alternative complement pathway activity (ACH50), agglutination titer, lysozyme activity and erythrocytes Cu/Zn-superoxide dismutase (SOD) activity of juvenile grouper fed for 18 weeks with diets containing different levels of zinc[1].

| Total zinc (mg/kg) | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 10.0 | 17.6 | 23.6 | 26.4 | 36.7 | 47.3 | 145.5 |
| Phagocytic index (n = 6) | 8.9 ± 7.8 | 15.6 ± 9.0 | 19.2 ± 9.4 | 18.6 ± 12.0 | 18.2 ± 7.4 | 21.0 ± 16.0 | 17.3 ± 7.2 |
| ACH50, U/ml (n = 3) | 121 ± 12 | 146 ± 17 | 160 ± 52 | 129 ± 50 | 157 ± 16 | 99 ± 55 | 88 ± 22 |
| Agglutination titer (n = 3) | 6.0 ± 1.0 | 6.3 ± 0.6 | 6.3 ± 0.6 | 5.7 ± 0.6 | 6.7 ± 0.6 | 8.3 ± 0.6 | 7.3 ± 2.0 |
| Lysozyme, U/ml (n = 3) | 139.0 ± 5.0 | 146.1 ± 5.0 | 135.5 ± 0.0 | 142.6 ± 10.0 | 142.6 ± 10.0 | 142.6 ± 0.0 | 139.0 ± 5.0 |
| Cu/Zn-SOD, U/mg protein (n = 3) | 162.5 ± 8.8 | 158.5 ± 19.3 | 127.0 ± 22.5 | 99.3 ± 10.6 | 155.0 ± 44.5 | 146.5 ± 14.3 | 121.3 ± 29.4 |

[1] Means in the same row with different superscript letters are significantly different ($P < 0.05$).

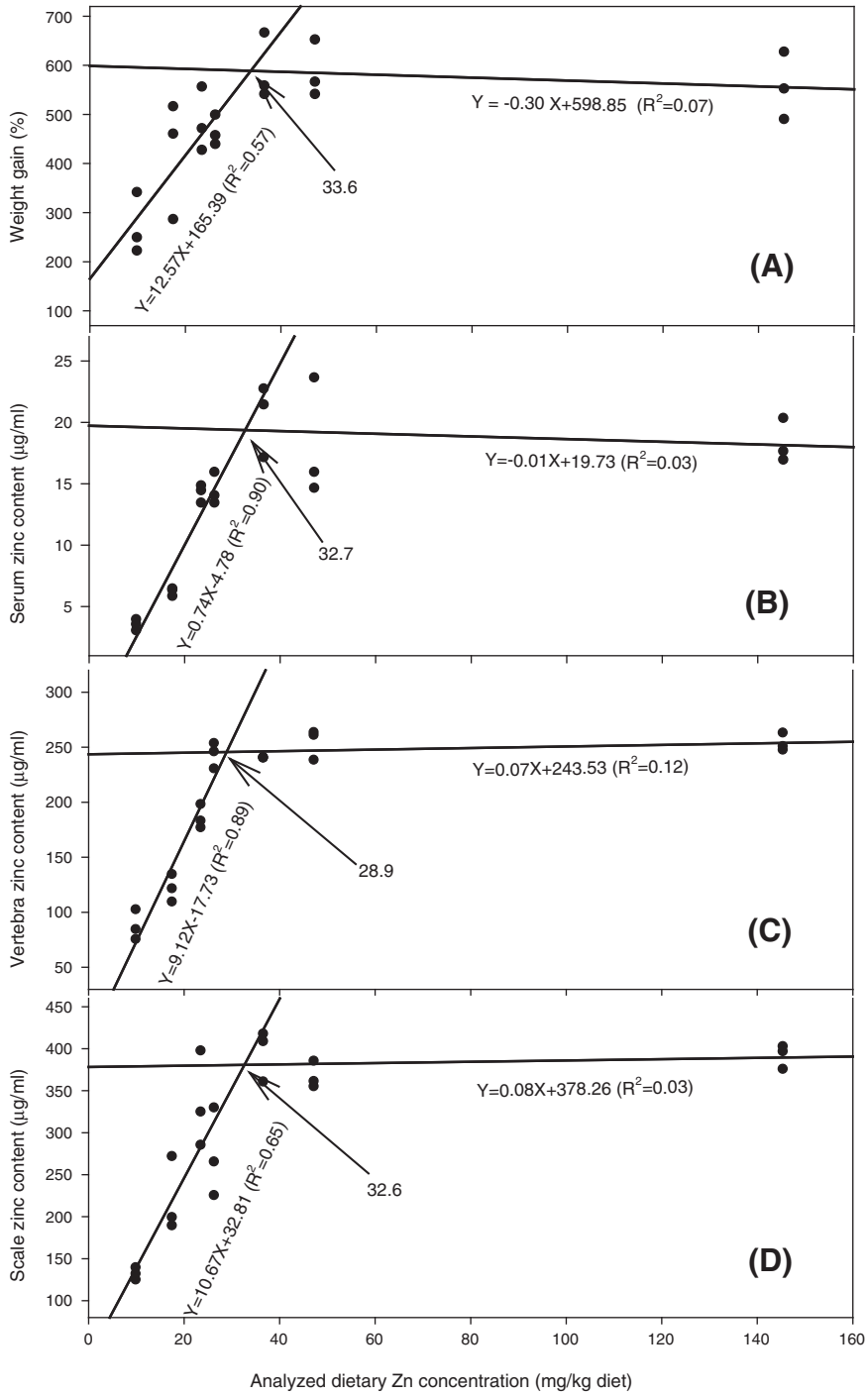


**Fig. 1.** Regressions of weight gain (A), serum zinc concentration (B), vertebra zinc concentration (C), and scale zinc concentration (D) values on analyzed dietary zinc concentration indicate breakpoints in the lines for juvenile grouper *Epinephelus malabaricus* fed diets containing various levels of zinc. Each point in tissue Zn concentrations represents the mean of five fish in one tank.

aquatic organisms, including phagocytic index, ACH50, agglutination titer, lysozyme activity and Cu/Zn-SOD activity. These results clearly demonstrated that the immunity of the grouper, as measured by the indicators examined in the present study, was not affected by the dietary Zn provision, which caused significant variations in body Zn abundance. In the present study, in addition to the residual Zn, the supplemental Zn was in the form of inorganic Zn. Although diets supplemented with Zn in organic forms (Mintrex, Novus) did not result in improved growth of turbot as compared to inorganic Zn (Ma et al., 2014), zinc methionine was found to be more potent than inorganic forms of Zn in enhancing resistance of channel catfish to pathogen infections (Paripatananont and Lovell, 1995b).

The lack of immune responses to Zn deficiency in the groupers is not unique. Scarpa and Gatlin (1992) were unable to identify the relationships between Zn deficiency and serum IgM, neutrophil abundance and bacterial challenge survival of channel catfish. Lim et al. (1996) found that dietary Zn levels did not affect the survival and macrophage phagocytic activity of channel catfish when challenged with pathogenic bacteria. In both studies, the Zn concentration in the deficiency diets was as low as 2 mg/kg and the fish were fed the deficiency diet for more than 8–16 weeks. Eid and Ghonim (1994) suggested that in order for a fish to develop Zn deficiency, the dietary Zn concentration might be 1 mg/kg or even less. It seems that the aquatic organisms will develop immunodeficiency signs only when these animals were subjected to severe Zn deficiency. More studies are needed to further explore the link between Zn deficiency and immune responses.

## 5. Conclusions

Broken-line model analyses of weight gain and tissue Zn concentrations showed that the juvenile grouper grew optimally when the diet contained a Zn level of 33.7 mg Zn/kg; zinc requirements that were estimated based on serum, vertebrate and scale Zn concentrations were 32.5, 28.9 and 32.6 mg Zn/kg, respectively. These results revealed that the dietary requirements of juvenile *E. malabaricus* for Zn ranged between 28.9 and 33.7 mg/kg, which is within the range reported for various fishes.

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