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Aquaculture 192 (2001) 67–84

Aquaculture

www.elsevier.nl/locate/aqua-online

Zinc methionine and zinc sulfate as sources of dietary zinc for juvenile abalone, *Haliotis discus hannai* Ino

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Received 31 January 2000; received in revised form 22 May 2000; accepted 22 May 2000

Abstract

A feeding experiment was conducted to determine the minimum dietary zinc requirement of juvenile abalone, *Haliotis discus hannai*, with zinc methionine (ZnMet) and zinc sulfate ($ZnSO_4 \cdot 7H_2O$) as the zinc sources and to compare the bioavailability of the two zinc sources using a premium quality diet based on casein–gelatin as the protein sources. Semipurified experimental diets containing graded levels of dietary zinc (5.6–84.6 mg zinc/kg) provided as either ZnMet or $ZnSO_4$ were fed to juvenile abalone in triplicate groups for 16 weeks. The results showed that the average weight gain rate (WGR, %), daily increment in shell length (DISL, μm/day), soft-body alkaline phosphatase activity (SBAKP, U/g protein) and soft-body zinc concentration (SB zinc, μg/g) of the abalone were significantly (ANOVA, $P < 0.01$) affected by dietary treatment, and responded in broken-line models to increases in dietary zinc levels from the two zinc sources. The requirements for dietary zinc using ZnMet and $ZnSO_4$ as the supplemental zinc sources, determined by broken-line regression analysis, on the basis of maximum WGR were 15.49 and 34.10 mg/kg, respectively, on maximum DISL were 15.16 and 33.99 mg/kg, respectively, on maximum SBAKP were 15.54 and 31.91 mg/kg, and on maximum SB zinc deposition were 17.75 and 34.29 mg/kg, respectively. The shell zinc concentration, as well as iron concentration in soft-body and shell of the abalone, however, was maintained relatively constant (ANOVA, $P > 0.05$) regardless of dietary treatment. Based on these results, a minimum requirement for dietary zinc was recommended to be 16–18 mg/kg from ZnMet, and 35 mg/kg from $ZnSO_4$. This experiment also showed that the bioavailability of dietary zinc with ZnMet was approxi-

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PII: S0044-8486(00)00435-X

mately three times as high as that of $ZnSO_4$ to *H. discus hannai* Ino.

Keywords: Dietary zinc; Requirement; Bioavailability; *Haliotis discus hannai*; Feeding and nutrition — mollusks

1. Introduction

Zinc is known to be an essential micronutrient both in plants and animals. Zinc is involved in various metabolic pathways. It serves as a specific cofactor of several enzymes. In addition, zinc is an integral part of about 20 metalloenzymes, such as alkaline phosphatase, alcohol dehydrogenase and carbonic anhydrase. Zinc is associated with prostaglandin metabolism and also may have a structural role in nucleoproteins (reviewed by Watanabe et al., 1997). Recent research on zinc–gene interactions has assigned a basic role for this element in controlling growth (Chesters, 1991). Normal zinc levels in freshwater (Spry et al., 1988) and seawater (Willis and Sunda, 1984) are known to be insufficient to meet the requirement of growing aquatic species. Hence, zinc is regarded as an essential nutrient in fish feeds (e.g. Lall, 1989; NRC, 1993; Wei et al., 1999) and shrimp feeds (e.g. Li et al., 1995). The zinc requirement of rainbow trout was found to be between 15 and 30 mg/kg diet (Ogino and Yang, 1978). Gatlin et al. (1991) reported that dietary zinc requirement of the red drum was between 20 and 25 mg/kg diet. Whereas, the requirement of Atlantic salmon for zinc is ≥ 67 mg/kg diet (Maage and Julshamn, 1993). All these requirements were determined with zinc sulfate ($ZnSO_4 \cdot 7H_2O$) as supplemental zinc source.

Amino acid-chelated zinc has been shown to have a higher absorption rate in the animal intestine than inorganic forms of zinc, such as zinc sulfate, zinc carbonate, and zinc oxide (Ashmead, 1992). Wedekind et al. (1992) reported that the bioavailability of zinc from zinc methionine (ZnMet) was greater than that of zinc sulfate ($ZnSO_4$) and the difference in bioavailability increased as complexity of the diet increased. The bioavailability of ZnMet relative to that of $ZnSO_4$ was 117% in a crystalline amino acid purified diet and 206% in a practical corn–soybean diet. Similar results have been reported by Paripatananont and Lovell (1995) in channel catfish. They found that the relative bioavailabilities of ZnMet, with $ZnSO_4$ as the standard, were 352% for weight gain and 305% for bone zinc deposition in fish fed an egg-white diet, and 482% for weight gain and 586% for bone zinc deposition in fish fed a soybean meal diet.

High dietary levels of zinc may, however, negatively affect the status of other elements, such as iron (Wekell et al., 1986). Unnecessarily high additions of zinc and other micronutrients also increase the price of feeds, as well as increase the input of minerals to the aquatic environment (Maage and Julshamn, 1993).

There is no information on the requirement of dietary zinc by any mollusks. Also, no information is available on the bioavailabilities of organic and inorganic sources of dietary zinc to these mollusk species. Declining abalone fisheries worldwide (Clavier, 1992; Farlinger and Campbell, 1992; Guzmán del Proó, 1992; Johnson et al., 1992; Nie, 1992; Parker et al., 1992; Prince and Shepherd, 1992; Schiel, 1992; Tarr, 1992; Tegner

et al., 1992) have accelerated the development of abalone aquaculture. *Haliotis discus hannai* is one of the most widely cultured and commercially important abalone species. Therefore, the objectives of this study were to determine the dietary zinc requirement of juvenile abalone, *H. discus hannai* Ino, with ZnMet and $ZnSO_4$ as the zinc sources and to compare the bioavailabilities of the two zinc sources using a premium quality diet based on casein–gelatin as the protein sources. This information will enable feed formulators to determine which zinc sources and what amount of dietary zinc should be used in the abalone diets.

2. Materials and methods

2.1. Feed formulation and manufacture

The basal diet formulation is given in Table 1. Casein and gelatin were used as protein sources. Crude protein level of the experimental diets was 28%, which is considered to be sufficient to maintain optimum growth of *H. discus hannai* (Mai et al., 1995b). Soybean oil and menhaden fish oil (1:1) were used as the lipid sources. Dietary

Table 1
Composition of the basal diet (dry weight basis)

Ingredient	Percent in diet
Casein (Sigma, St. Louis, MO, USA)	25.00
Gelatin (Sigma)	6.00
Dextrin (Shanghai Chemical, Shanghai, China)	33.50
Sodium alginate (Shanghai Chemical)	20.00
SO/MFO (Food grade)[a]	3.50
Choline chloride (Shanghai Chemical)	0.50
Carboxymethylcellulose (Shanghai Chemical)	5.00
Zinc-free mineral mix[b]	4.50
Vitamin mix[c]	2.00
Proximate analysis (means of triplicate)	
Crude protein (%)	28.40
Crude lipid (%)	3.48
Ash (%)	8.78
Gross energy (kJ/g)[d]	17.18
Zinc (mg/kg)	5.60

[a] Soybean oil and menhaden fish oil (1:1) with 0.001% ethoxyquin.

[b] Zn-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, 20.0 g; $Ca(H_2PO_4)_2 \cdot H_2O$, 8.0 g; Fe-citrate, 1.0 g; $MnSO_4 \cdot H_2O$, 64.8 mg; $CuSO_4 \cdot 5H_2O$, 12.4 mg; $CoCl_2 \cdot 6H_2O$, 0.4 mg; KIO_3, 1.2 mg; Na_2SeO_3, 0.4 mg.

[c] Vitamin mix, each 1000 g of diet contained: thiamin HCl, 120 mg; riboflavin, 100 mg; folic acid, 30 mg; PABA, 400 mg; pyridoxine HCl, 40 mg; niacin, 800 mg; Ca pantothenate, 200 mg; inositol, 4000 mg; ascorbic acid, 4000 mg; biotin, 12 mg; vitamin E, 450 mg; menadione, 80 mg; B12, 0.18 mg; retinol acetate, 100,000 IU; cholecalciferol, 2000 IU; ethoxyquin, 400 mg.

[d] Estimated with an XYR-1 bomb calorimeter.

lipid level was 3.5%, which was sufficient to support optimum growth and provide adequate essential fatty acids (EFA) for abalone (Mai et al., 1995a). The compositions of vitamin and mineral mixtures were modified from those used by Uki et al. (1985). The casein–gelatin-based diet contained 5.60 ± 0.79 mg/kg of intrinsic zinc. This semipurified formulation was employed because previous experiences in our laboratory indicated limited acceptance of egg white-based purified diets by juvenile abalone. The basal diet was supplemented with 0, 5, 10, 20, 30, 40, and 80 mg of zinc/kg dry diet from either zinc methionine (ZnMet) (Feed Additive, Ministry of Chemistry Industry of China, Jinan) or zinc sulfate ($ZnSO_4 \cdot 7H_2O$) (Sigma, St. Louis, MO, USA). Final zinc concentrations in the experimental diets ($n = 3$) were found to be: 5.6 ± 0.79, 11.2 ± 0.87, 14.8 ± 0.96, 26.1 ± 1.22, 34.9 ± 0.86, 43.3 ± 1.13, and 83.9 ± 1.48 mg/kg from ZnMet, and 5.6 ± 0.79, 10.7 ± 0.69, 15.1 ± 0.48, 25.2 ± 0.76, 34.7 ± 0.58, 45.3 ± 1.06, and 84.6 ± 1.25 mg/kg from $ZnSO_4$ as determined by ICP-AES (Shearer, 1984).

Procedures for diet preparation were modified from those described by Mai et al. (1995a,b). Casein, gelatin and some minerals were ground individually using a Pascal Mill and then passed through a mesh with 125-μm pore size. Dry ingredients were weighed on an electronic balance and thoroughly mixed. After adding water (about 120%, v/w) to the mechanically mixed ingredients containing 20% sodium alginate, a paste was made. The paste was shaped into 0.5-mm thick sheets, which were cut into 1-cm^2 flakes. The flakes were dipped into an aqueous solution of $CaCl_2$ (5%, w/v) for 1 min. By this treatment, sodium alginate was converted to an insoluble calcium alginate gel, in which the nutrients were bound (Uki and Watanabe, 1992). The surplus solution was drained, and then the flakes were sealed in a sample bag and stored at −20°C until use.

2.2. Animal rearing

Juvenile abalone, *H. discus hannai* used in this experiment was derived from a spawning in June 1998, at Mashan Fisheries, Shandong, China. Before trial, shell length was measured with calipers to the nearest 0.02 mm and the animals were weighed to the nearest 0.01-g using an electronic balance.

A series of acrylic cages (1.5 × 1.0 × 0.5 m) were put into a rectangle cement tank (6 × 2 × 1.5 m). Animals were kept in plastic baskets (20 × 20 × 20 cm). Each rearing unit (basket) was stocked with 25 abalone juveniles (mean weight 0.74 ± 0.01 g; mean shell length 16.41 ± 0.04 mm). There were 13 treatments, and each treatment was conducted in three replicates. Thirty-nine baskets were assigned to the acrylic cages using a completely randomized design. Seawater pumped from the coast adjacent to the farm passed through sand filters (filtered to 30 μm by primary sand filters, followed to 10 μm by secondary composite sand filters) into the tank continuously at a rate of 145 ± 9 l/min. During the experimental period, water temperature ranged from 18.5–22.0°C, salinity 30–34, pH 7.6–7.9. Dissolved oxygen was not less than 7.0 mg/l, and there were negligible levels of free ammonia and nitrite. Zinc concentration in the water flowing into the rearing system was 10.0 ± 2.3 μg/l as determined by ICP-AES ($n = 3$).

Prior to initiation of the experiment, the abalone underwent a 2-week conditioning period during which they readily adjusted to a zinc-depleted casein–gelatin-based diet (Table 1) and standardized environmental conditions. The feeding trial was run for 16 weeks. Abalone were hand-fed with the test diets at a rate equaling 5–10% of wet body weight/day, once daily at 17:00. Every morning, uneaten feed and feces were cleaned to maintain water quality.

2.3. Sample collection and analyses

At the termination of the experiment, animals were not fed for 3 days, then all abalone were removed from the cages, weighed, measured and counted. Then, 15 abalone from each replicate were frozen (-70°C) for subsequent analyses. Growth was expressed as weight gain rate (WGR, %) and daily increment in shell length (DISL, μm/day). The calculation formulae are as follows:

$$\mathrm{WGR}(\%) = \left[(W_t - W_i)/W_i\right] \times 100$$

$$\mathrm{DISL} = \left[(\mathrm{SL}_t - \mathrm{SL}_i)/t\right] \times 1000$$

where W_t, W_i are final and initial mean weight (g), respectively; SL_t, SL_i are final and initial mean shell length (mm), respectively; t is the feeding trial period (day).

Proximate analyses of soft-body samples to determine protein, lipid, ash and moisture levels were conducted using standard procedures (AOAC, 1984).

Thawed soft bodies were weighed, and homogenized in cold (4°C) 0.01 M Tris buffer (pH 7.5) at a ratio of 1:20 (w/v). The crude extract was obtained by centrifuging the homogenate at $13{,}500 \times g$ for 20 min at 4°C, and filtering the supernatant. Soft body alkaline phosphatase (SBAKP) activity in the crude extract was determined spectrophotometrically using a ρ-nitrophenyl-phosphate substrate assay (Tietz, 1986). Protein was estimated by a modification of the Lowry procedure (Hartee, 1972) with bovine serum albumin as the calibration standard. SBAKP activity was expressed as specific activity (U/g protein), where one unit (U) is equal to the amount of enzyme necessary to produce 1 μmol of nitrophenol (from ρ-nitrophenyl-phosphate)/min at 37°C. Elemental analyses of the shells and soft bodies of the abalone were modified from the method described by Shearer (1984). The shell samples were digested in a mixture of equal parts of hydrochloric acid (37%, ACS reagent) and nitrite acid (70%, ACS reagent) at a ratio of 1:20 (w/v). The soft body samples were digested in perchloric acid ($HClO_4$, 70%, ACS reagent) at a ratio of 1:20 (w/v). Then, the digests were appropriately diluted with Milli-Q water within the analytical capabilities of the ICP atomic emission spectrophotometer (JY 70plus, Jobin Yvon). Elemental concentrations of the samples are expressed on a wet-weight basis as recommended by Shearer (1984).

2.4. Leaching

A leaching test for zinc was carried out according to the method used by Coote et al. (1996). Pre-weighed diet was placed onto 100-μm mesh screens and allowed it to settle to the bottom of experimental cages without abalone. Temperature and flow rate were

adjusted to match those of the experiment, the values being 20 ± 0.8°C, and about 0.5-l/min per cage, respectively. At the end of allotted time (0, 6, and 12 h, respectively), the remaining diet was removed from the cages and dried overnight at 60°C in an oven. Dried diet was submitted for analysis of total zinc with an ICP-atomic emission spectrophotometer.

2.5. Statistical analysis

Data from each treatment were subject to one-way ANOVA. When overall differences were significant at less than 5% level, Tukey's test was used to compare the mean values between individual treatments. Statistical analysis was performed using the STATISTICA™ package. Dietary zinc requirements of juvenile abalone were estimated by broken-line regression analysis (Robbins et al., 1979; Robbins, 1986). The linear segments of the regression lines below the breakpoints were used to compare the bioavailability of dietary zinc from ZnMet with that from $ZnSO_4$ by deriving the ratio of the slopes of the lines (Forbes and Parker, 1977; Paripatananont and Lovell, 1995).

3. Results

3.1. Leaching

The results of the leaching test with experimental diets is illustrated in Fig. 1. The zinc content of the diets supplemented with the two zinc sources all declined during the test period. After 6 h in seawater, the remaining zinc content of the diets ranged from 4.78 to 52.45 mg/kg for ZnMet diets (Fig. 1A), and from 4.78 to 50.08 mg/kg for $ZnSO_4$ diets (Fig. 1B). After 12 h of immersion in seawater, the dietary zinc content ranged from 3.96 to 29.35 mg/kg for ZnMet diets (Fig. 1A), and from 3.96 to 31.05 for $ZnSO_4$ diets (Fig. 1B). There was a similar leaching rate between the two zinc sources ($P > 0.1$, as determined by analysis of co-variance). After 6 h of immersion in seawater, the leached zinc accounted for approximately 40% of the total zinc in the diets for the two sources of zinc, and this value increased to approximately 60% after 12 h of immersion in seawater.

3.2. Survival and growth

There were no significant differences in survival of abalone fed the dietary treatments (ANOVA, $P > 0.05$), which ranged from 94.7% to 100.0% for both zinc sources (Tables 2 and 3). The mean weight gain rate (WGR, %) and daily increment in shell length (DISL, μm/day) of the animals, however, were significantly affected by the various levels of dietary zinc from the two zinc sources (Tables 2 and 3). WGR ranged from 53% to 143% for ZnMet (Table 2), and from 53% to 136% for $ZnSO_4$ (Table 3). DISL ranged from 62 to 89 μm/day for ZnMet (Table 2), and from 62 to 87 μm/day for $ZnSO_4$ (Table 3). Both WGR and DISL responded in broken-line models to increases in dietary zinc levels from both sources of zinc (Figs. 2 and 3). The breakpoint in the

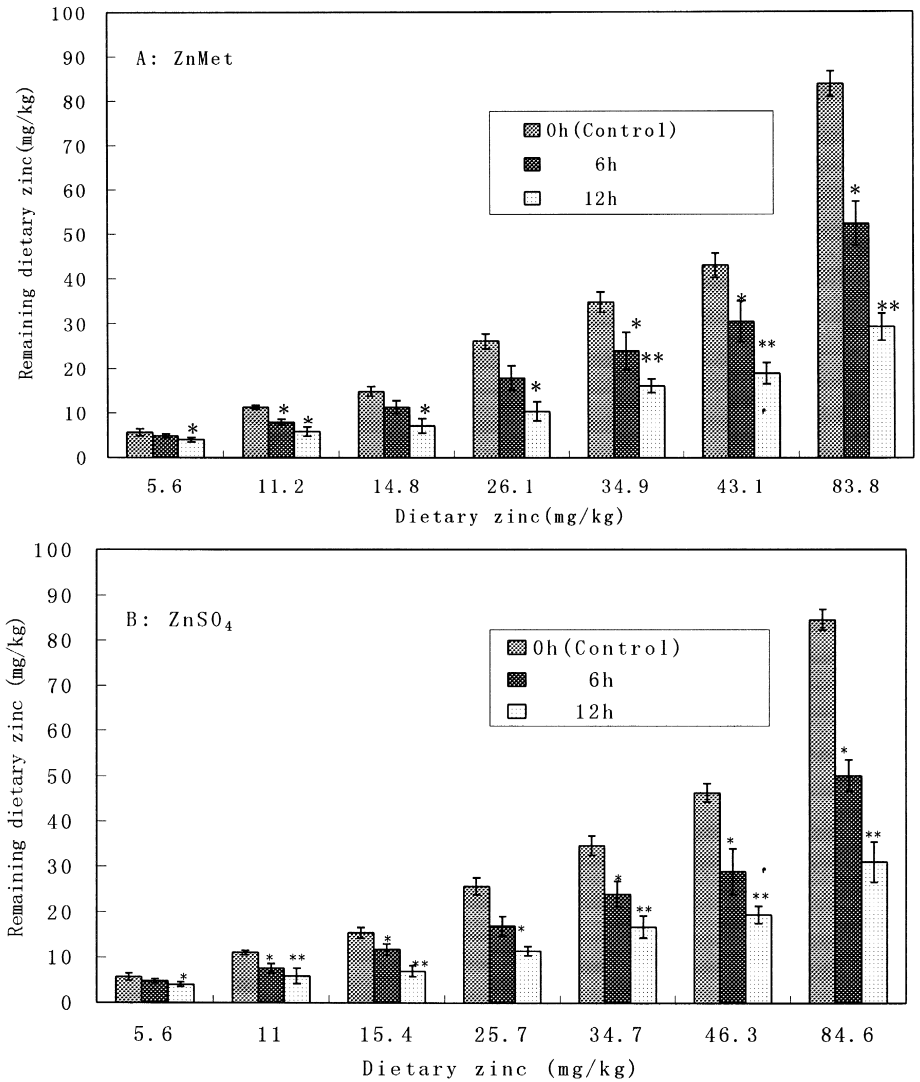

Fig. 1. Changes of zinc content in the diets containing various levels of supplemental zinc to the basal diet from either ZnMet (A) or ZnSO4 (B) with increasing immersion time (0, 6 and 12 h, respectively) in seawater. Error bars are the SD, values significantly different (ANOVA, Tukey's test) from the controls (0 h) are indicated with asterisks (* for $P < 0.05$ and ** for $P < 0.01$).

regression line, which was considered to be the minimum dietary level for optimum response, was 15.5 mg zinc/kg diet for WGR, and that was 15.2 mg zinc/kg diet for DISL when using ZnMet as the zinc source (Fig. 2). However, the breakpoint values for WGR and DISL were much higher when using $ZnSO_4$ as the zinc source compared to ZnMet, and reached 34.1 and 34.0 mg zinc/kg diet, respectively (Fig. 3). On the basis

Table 2
Weight gain rate (WGR), daily increment in shell length (DISL) and survival of abalone fed graded levels of dietary zinc from zinc methionine (ZnMet) for 16 weeks

Supplemental zinc (mg/kg)	Dietary zinc (mg/kg)	Initial shell length (mm)	Initial weight (g)	Final shell length (mm)	Final weight (g)	WGR (%)	DISL (μm/day)	Survival (%)
0	5.6	16.41	0.74	23.27[a]	1.13[a]	53.2[a]	62.3[a]	94.7
5	11.2	16.39	0.73	24.71[b]	1.49[b]	102.6[b]	76.6[b]	98.7
10	14.8	16.44	0.75	26.00[c]	1.76[c]	137.0[c]	86.9[c]	100.0
20	26.1	16.48	0.75	26.15[c]	1.79[c]	138.8[c]	87.9[c]	100.0
30	34.9	16.41	0.73	26.06[c]	1.76[c]	140.3[c]	87.7[c]	100.0
40	43.1	16.37	0.73	26.21[c]	1.78[c]	142.8[c]	89.4[c]	98.7
80	83.9	16.39	0.74	25.91[c]	1.74[c]	137.0[c]	86.5[c]	100.0
ANOVA								
Pooled SEM		0.1	0.03	0.2	0.05	8.3	5.2	4.5
F value		0.36	0.62	75.05	110.27	89.01	70.51	1.67
P value		0.89	0.71	0.00	0.00	0.00	0.00	0.20

Means in the same column sharing a common superscript letter were not significantly different ($P > 0.05$) as determined by Tukey's test.

Table 3
Weight gain rate (WGR), daily increment in shell length (DISL) and survival of abalone fed graded levels of dietary zinc from zinc sulfate ($ZnSO_4 \cdot 7H_2O$) for 16 weeks

Supplemental zinc (mg/kg)	Dietary zinc (mg/kg)	Initial shell length (mm)	Initial weight (g)	Final shell length (mm)	Final weight (g)	WGR (%)	DISL (μm/day)	Survival (%)
0	5.6	16.41	0.74	23.27[a]	1.13[a]	53.2[a]	62.3[a]	94.7
5	10.7	16.36	0.72	23.67[ab]	1.24[a]	71.8[ab]	66.5[ab]	98.7
10	15.1	16.47	0.75	24.48[bc]	1.43[b]	90.7[b]	72.8[b]	100.0
20	25.2	16.57	0.76	25.19[cd]	1.60[c]	112.2[c]	78.4[c]	98.7
30	34.7	16.33	0.73	25.87[d]	1.72[cd]	136.2[d]	86.6[d]	100.0
40	45.3	16.46	0.75	25.83[d]	1.74[cd]	132.4[d]	85.2[d]	98.7
80	84.6	16.41	0.74	25.80[d]	1.75[d]	135.8[d]	86.0[d]	100.0
ANOVA								
Pooled SEM		0.1	0.03	0.2	0.03	9.4	4.8	4.9
F value		0.80	1.49	32.43	77.27	64.40	35.26	1.26
P value		0.59	0.25	0.00	0.00	0.00	0.00	0.34

Means in the same column sharing a common superscript letter were not significantly different ($P > 0.05$) as determined by Tukey's test.

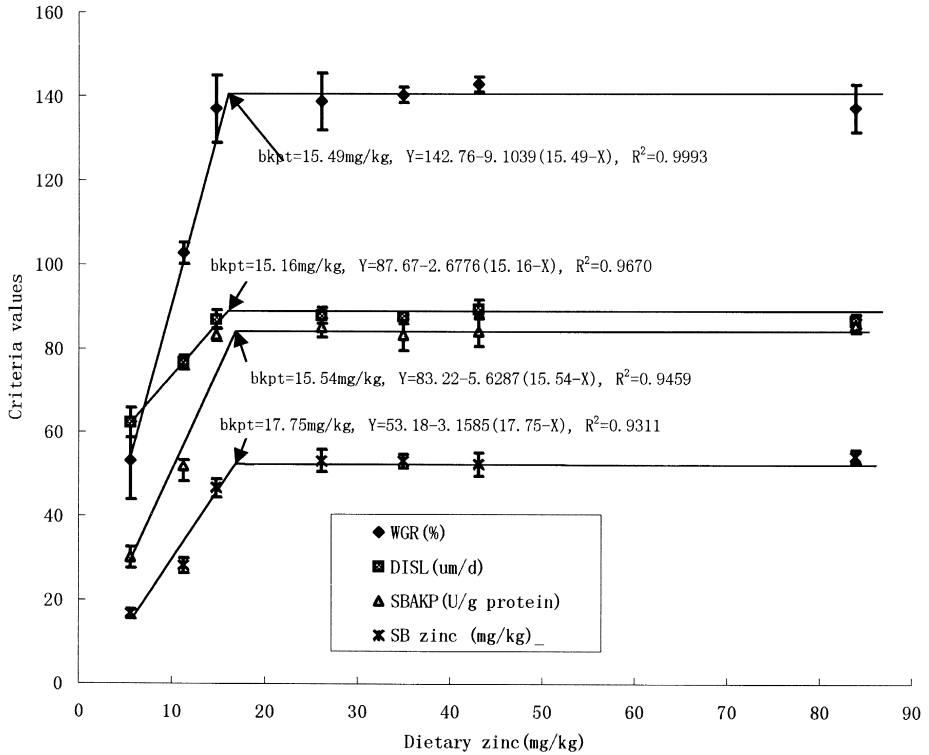

Fig. 2. Regression of weight gain rate (WGR, %), daily increment in shell length (DISL, μm/day), soft-body alkaline phosphatase activity (SBAKP, U/g protein) and soft-body zinc concentration (mg/kg, wet-weight basis) on dietary zinc levels and breakpoints (bkpt) in the lines for juvenile abalone fed diets containing graded levels of zinc methionine (ZnMet) for 16 weeks. The term "Criteria values" represents the values of selected criteria, including WGR, DISL, SBAKP and SB zinc.

of WGR or DISL, the bioavailability of dietary zinc mainly from ZnMet to abalone was significantly higher ($P < 0.01$, as determined by analysis of co-variance) than that from $ZnSO_4$. The ratios of the slopes of the broken-line equations were 3.28 (9.1039/2.774) for WGR and 3.27 (2.6776/0.8189) for DISL with ZnMet as zinc source compared to $ZnSO_4$ (Figs. 2 and 3). Thus, the bioavailability of dietary zinc mainly from ZnMet seemed to be about three times as high as that mainly from $ZnSO_4$.

3.3. Carcass composition and SBAKP activity

There were no significant differences ($P > 0.05$) in carcass composition of the abalone fed various levels of dietary zinc from the two zinc sources (Table 4). Soft body alkaline phosphatase (SBAKP) activity ranged from 30.0 to 85.4 U/g protein for abalone fed diets containing graded levels of supplemental zinc from ZnMet, and from 30.0 to 86.2 U/g protein for abalone fed diets containing graded levels of supplemental zinc from $ZnSO_4$ (Table 4). Enzyme activity responded in broken-line models to

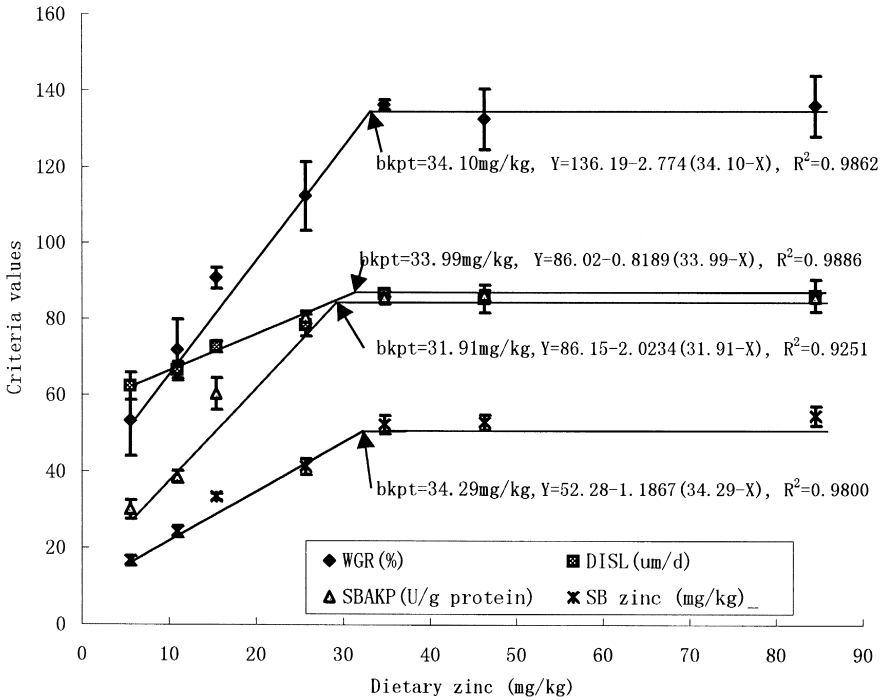

Fig. 3. Regression of weight gain rate (WGR, %), daily increment in shell length (DISL, μm/day), soft-body alkaline phosphatase activity (SBAKP, U/g protein) and soft-body zinc concentration (mg/kg, wet-weight basis) on dietary zinc levels and breakpoints (bkpt) in the lines for juvenile abalone fed diets containing graded levels of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) for 16 weeks. The term "Criteria values" represents the values of selected criteria, including WGR, DISL, SBAKP and SB zinc.

increases in dietary zinc levels with both sources of zinc. The breakpoints in the regression lines were 15.5-mg zinc/kg diet for ZnMet (Fig. 2), and 31.9-mg zinc/kg diet for $ZnSO_4$ (Fig. 3). On the basis of SBAKP activity, the bioavailability of dietary zinc mainly from ZnMet to abalone was significantly higher ($P < 0.01$, as determined by analysis of co-variance) than that from $ZnSO_4$.The bioavailability of dietary zinc mainly from ZnMet in relation to that mainly from $ZnSO_4$ was 278% ($100 \times 5.6278/2.0234$).

3.4. Elemental concentrations

The levels of soft body ash, zinc, and iron are shown in Table 5. After 16 weeks of feeding trial, no significant differences ($P > 0.05$) were observed in the levels of soft body ash and iron among dietary treatments (Table 5). The zinc content, however, was significantly affected ($P < 0.01$) by the various levels of dietary zinc from the two zinc sources. The zinc content ranged from 16.6 to 54.6 μg/g for $ZnSO_4$, and from 16.6 to 54.3 μg/g for ZnMet. Zinc content also responded in a broken-line model to increases in dietary zinc levels with both sources of zinc (Figs. 2 and 3). The breakpoints in the

Table 4
Carcass composition and alkaline phosphatase (SBAKP) activity in abalone fed graded levels of supplemental zinc from $ZnSO_4$ or ZnMet for 16 weeks*

Supplemental zinc (mg/kg)**	Moisture (%)		Protein (%)		Lipid (%)		SBAKP (U/g protein)	
	$ZnSO_4$	ZnMet	$ZnSO_4$	ZnMet	$ZnSO_4$	ZnMet	$ZnSO_4$	ZnMet
0	77.52	77.52	53.41	53.41	7.17	7.17	30.0[a]	30.0[a]
5	77.14	76.67	53.56	53.57	7.36	7.21	38.5[a]	51.8[b]
10	77.19	77.25	53.44	53.52	7.28	7.30	60.3[b]	83.3[c]
20	76.76	77.20	52.86	53.74	7.45	7.29	80.4[c]	85.1[c]
30	77.29	76.81	53.48	53.52	7.48	7.24	85.5[c]	83.2[c]
40	77.36	77.15	53.29	53.96	7.29	7.33	86.2[c]	84.4[c]
80	77.13	76.88	53.87	53.92	7.39	7.39	85.5[c]	85.4[c]
ANOVA								
Pooled SEM	1.0	0.9	0.4	0.3	0.2	0.1	4.2	4.0
F value	0.94	1.65	0.70	0.45	0.61	0.83	238.77	123.74
P value	0.52	0.26	0.66	0.82	0.72	0.58	0.00	0.00

Means in the same column sharing a common superscript letter were not significantly different ($P > 0.05$) as determined by Tukey's test.

* Values are means of three groups of abalone, with eight abalone/group ($n = 3$) for determining moisture, protein and lipid, and three abalone/group ($n = 3$) for measuring SBAKP.

** The basal diet contained 5.60 mg of zinc/kg diet, and the measured total dietary zinc levels are the same as those in Tables 2 and 3.

Table 5
Ash and selected elemental concentrations in the soft bodies of abalone fed various levels of supplemental zinc from $ZnSO_4$ or ZnMet for 16 weeks*

Supplemental zinc (mg/kg)**	Ash (%)#		Zinc (μg/g)##		Iron (μg/g)##	
	$ZnSO_4$	ZnMet	$ZnSO_4$	ZnMet	$ZnSO_4$	ZnMet
0	11.50	11.50	16.56[a]	16.56[a]	486	486
5	11.49	11.54	24.15[b]	28.04[b]	515	499
10	11.52	11.51	33.31[c]	46.56[c]	516	520
20	11.48	11.53	41.25[d]	53.18[c]	521	506
30	11.49	11.46	52.28[e]	53.12[c]	504	512
40	11.59	11.56	52.81[e]	52.40[c]	509	504
80	11.48	11.59	54.56[e]	54.27[c]	522	524
ANOVA						
Pooled SEM	0.9	1.0	1.3	1.1	21.7	18.5
F value	0.22	0.48	127.16	115.33	0.72	0.83
P value	0.96	0.80	0.00	0.00	0.65	0.58

Means in the same column sharing a common superscript letter were not significantly different ($P > 0.05$) as determined by Tukey's test.

* Values are means of three groups of abalone,with the soft bodies of four abalone/group.($n = 3$).

** The basal diet contained 5.60 mg of zinc/kg diet, and the measured total dietary zinc levels are the same as those in Tables 2 and 3.

Dry-weight basis.

Wet-weight basis.

Table 6
Ash and selected elemental concentrations in the shells of abalone fed various levels of supplemental zinc from $ZnSO_4$ or ZnMet for 16 weeks *

Supplemental zinc (mg/kg) * *	Ash (%)#		Zinc (μg/g)##		Iron (μg/g)##	
	$ZnSO_4$	ZnMet	$ZnSO_4$	ZnMet	$ZnSO_4$	ZnMet
0	74.54	74.54	28.20	28.20	117	117
5	74.51	74.47	29.56	28.33	126	121
10	74.64	74.58	28.64	29.72	123	120
20	75.58	74.52	29.63	30.51	121	126
30	74.49	74.49	30.24	30.08	118	122
40	74.68	74.55	29.11	30.97	125	127
80	74.54	74.52	28.44	29.23	130	123
ANOVA						
Pooled SEM	0.1	0.2	1.0	1.2	8.6	9.1
F value	0.34	0.21	0.67	1.72	0.44	0.31
P value	0.89	0.96	0.68	0.25	0.83	0.91

* Values are means of three groups of abalone, with the shells of 4 abalone/group ($n = 3$).
* * The basal diet contained 5.60 mg zinc/kg diet, and the measured total dietary zinc levels are the same as those in Tables 2 and 3.
Dry-weight basis.
Wet-weight basis.

regression lines were 17.8-mg zinc/kg diet for ZnMet (Fig. 2), and 34.3-mg zinc/kg diet for $ZnSO_4$ (Fig. 3). On the basis of SB zinc deposition, the bioavailability of dietary zinc mainly from ZnMet to abalone was significantly higher ($P < 0.01$, as determined by analysis of co-variance) than that from $ZnSO_4$. The bioavailability of zinc from

Table 7
The dietary zinc requirements on the basis of weight gain rate (WGR, %), daily increment in shell length (DISL, μm/day), soft body alkaline phosphatase (SBAKP, U/g protein) and soft body zinc concentration (SB zinc, μg/g) determined with ZnMet and ZnSO4 as the zinc sources and the relative bioavailability (%) of dietary zinc with ZnMet as compared with $ZnSO_4$ in juvenile abalone

Criteria	Zinc sources	Broken-line equation	Breakpoints (mg/kg)[a, *]	Requirements * (mg/kg)	Relative bioavailability (%)[b]
WGR	ZnMet	$Y = 142.76 - 9.1039(15.49 - X)$	15.49 ± 0.3	15.5 ± 0.3	328
	$ZnSO_4$	$Y = 136.19 - 2.774(34.10 - X)$	34.10 ± 0.7	34.1 ± 0.7	
DISL	ZnMet	$Y = 87.67 - 2.6776(15.16 - X)$	15.16 ± 0.2	15.2 ± 0.2	327
	$ZnSO_4$	$Y = 86.02 - 0.8189(33.99 - X)$	33.99 ± 0.7	34.0 ± 0.7	
SBAKP	ZnMet	$Y = 83.22 - 5.6287(15.54 - X)$	15.54 ± 0.2	15.5 ± 0.2	278
	$ZnSO_4$	$Y = 86.15 - 2.0234(31.91 - X)$	31.91 ± 0.6	31.9 ± 0.6	
SB zinc	ZnMet	$Y = 53.18 - 3.1585(17.75 - X)$	17.75 ± 0.3	17.8 ± 0.3	266
	$ZnSO_4$	$Y = 52.28 - 1.1867(34.29 - X)$	34.29 ± 0.7	34.3 ± 0.7	

* Values are means ± SEM ($n = 3$) as determined by broken-line regression analysis (Robbins et al., 1979).
[a] Breakpoint in the regression line.
[b] The ratio of the slope of ZnMet broken-line equation to the slope of $ZnSO_4$ broken-line equation × 100.

ZnMet in relation to that from $ZnSO_4$ was 266% ($100 \times 3.1585/1.1867$). Both the ash content and the selected elemental concentrations in the shells were relatively constant ($P > 0.05$) regardless of dietary treatment (Table 6).

The dietary zinc requirements for different criteria determined with ZnMet and $ZnSO_4$ and the relative bioavailabilities of ZnMet compared to $ZnSO_4$ in juvenile abalone are summarized in Table 7.

4. Discussion

The diets used in this experiment supported satisfactory abalone growth. After 16 weeks of feeding, all groups which obtained sufficient dietary zinc from the two zinc sources grew well both in mean WGR and in mean DISL, in comparison to those reported by other authors (e.g. Uki et al., 1985; Uki and Watanabe, 1992; Mai et al., 1995a,b; Mai, 1998).

To our knowledge, there is only one published paper pertaining to the dietary mineral nutrition of abalone (*H. laevagata*) (Coote et al., 1996). This is probably in part due to the problems associated with incredible leaching of minerals added to soft moist diets. In the present experiment, the special feed manufacturing technology was adopted so as to improve the water stability of the feed. The leaching of supplemental zinc from the basal diet, however, was still high (Fig. 1). The leaching trials indicated that the remaining dietary zinc from the two zinc sources was approximately 60% of the total dietary zinc after 6 h of immersion in seawater, and this value decreased to 30–40% after 12 h of immersion. We observed the fact that the digestive tracts of most abalone were full of food within 2 h of feeding with the premium quality diets (Mai et al., 1998). This, together with the fact that a series of criteria responded in broken-line models to increases in dietary zinc levels with the two zinc sources makes the requirement of dietary zinc of juvenile abalone recommended in the present study acceptable. If the water stability of dietary zinc can be further improved, however, the requirement of dietary zinc for this species may be further reduced to a certain extent.

In the present study, the WGR and DISL were the two responsive parameters to dietary zinc levels from both ZnMet and $ZnSO_4$, and responded in broken-line models to increases in dietary zinc levels. Significantly depressed growth was noticed after 16 weeks of feeding abalone low-zinc diets. Impaired growth also has been observed in fishes fed low-zinc diets (Ogino and Yang, 1978, 1979; Gatlin and Wilson, 1983; Gatlin et al., 1991; Paripatananont and Lovell, 1995; Wei et al., 1999). The present result indicates that supplementation of zinc to the basal diet is necessary to obtain normal growth of abalone, *H. discus hannai*.

The activity of the zinc-containing enzyme, alkaline phosphatase, in soft-body was strongly influenced by dietary zinc concentration from the two zinc sources. This implies that the abalone did experience zinc deficiency, which would lead to depressed enzyme activity. Thus, the alkaline phosphatase activity was a useful criterion in estimating dietary zinc requirements and evaluating bioavailability of zinc for *H. discus hannai*.

Many studies examining the dietary elemental requirements of aquatic species have shown that depressed whole-body or tissue levels of essential elements could result from insufficient dietary intake (Lovell, 1978; Ogino and Yang, 1978, 1979; Gatlin et al., 1982; Wilson et al., 1982; Paripatananont and Lovell, 1995). Baker (1986) also indicated that studies on the mineral requirements of animals should include measurement of body stores of the test element. Mineral analyses at the end of the feeding trial indicated that soft-body zinc concentrations of the abalone increased linearly until dietary zinc reached 17.8 mg/kg for ZnMet (Fig. 2), and 34.3 mg/kg for $ZnSO_4$ (Fig. 3). The reduced zinc reserves were becoming depleted and deficiency signs would most likely become apparent if those diets were fed for an extended period of time. Therefore, the soft-body zinc concentration was also a responsive criterion for estimating the dietary zinc requirement of abalone. However, similar responses were not observed in shell zinc concentrations of the abalone. This suggests that shell zinc deposition of the abalone was not a useful criterion for determining the zinc requirement of the abalone, especially when the experimental duration was not long enough. Shellfish have special formation mechanisms for biomineralization of their hard tissues. Sakai (1980) found that the accumulation of organic acids in the rearing water could lead to severe shell erosion in young abalone, and then cause the shell to split along the respiratory apertures. Chen (1989) reported that there was a marked depression in calcium and zinc concentration in split *H. diversicolor supertexta*. These results, along with the data obtained in the present study, imply that the rearing water quality, such as pH, perhaps plays a more significant role than the dietary mineral concentration in shell mineralization and shell mineral deposition of abalone.

Many studies with fish have shown elevated iron levels in zinc deficiency (Ogino and Yang, 1978; Wekell et al., 1986; Spry et al., 1988). The zinc deficiency sign such as depressed growth occurred in this experiment, but no elevated iron levels were observed either in the shells or in the soft bodies from the abalone fed the low-zinc diets with ZnMet or $ZnSO_4$. The interaction among minerals such as zinc–iron should be further studied in mollusks.

The minimum level of dietary zinc for juvenile abalone varied with zinc sources and criteria used (Table 7). Data of growth, alkaline phosphatase and soft-body zinc concentration showed that about 16–18 mg/kg of dietary zinc from ZnMet could maintain optimum responses. However, when using $ZnSO_4$ as the supplemental zinc source, the minimum level of dietary zinc increased to 32–35 mg/kg. We therefore, estimate that the dietary zinc requirement of juvenile abalone is 16–18 mg/kg when using ZnMet as zinc. This estimated requirement is higher than that for channel catfish (6.58 mg zinc/kg; Paripatananont and Lovell, 1995). Using $ZnSO_4$ as the supplemental zinc sources, the dietary zinc requirement of the abalone was found to be 32–35 mg/kg. This value is higher than those reported for rainbow trout (15–30 mg/kg; Ogino and Yang, 1978), carp (15–30 mg/kg; Ogino and Yang, 1979), channel catfish (20 mg/kg; NRC, 1993), tilapia (20 mg/kg; McClain and Gatlin, 1988) and red drum (20–25 mg/kg; Gatlin et al., 1991).

Data from the present study indicate that the juvenile abalone required approximately three times as much zinc in the inorganic form as in organic form. The relative bioavailability values of ZnMet to $ZnSO_4$ for weight gain rate, 328%, daily increment in

shell length, 327%, alkaline phosphatase activity, 278%, and soft-body zinc deposition, 266%, accurately described the differences in the two sources of zinc. These results agree with those reported by Paripatananont and Lovell (1995). They indicated that the relative bioavailabilities of ZnMet, with $ZnSO_4$ as the standard, were 352% for weight gain and 305% for bone zinc deposition with egg-white diets.

The nutritional value of dietary mineral sources depends not only upon their content in the feedstuff but also upon the bioavailability of the element to the animal (Paripatananont and Lovell, 1997). Studies with mammals have shown that chelation of minerals to amino acids may increase their absorption rate in the intestine (Ashmead, 1992). Ashmead (1992) indicated that the higher bioavailability of amino acid-bound trace elements to animals is because chelation protects the element from forming insoluble complexes in the digestive tract and facilitates zinc transport across the intestinal mucosa. He also suggested that the chelate could remain intact until it reaches the site in the body where the element is needed. The results obtained in this study clearly indicated that the bioavailability of ZnMet is much higher than that of $ZnSO_4$ to juvenile abalone, *H. discus hannai*. Thus, in the formulation of diets for the abalone, the dietary allowance of zinc could be reduced by using the chelated-zinc instead of the inorganic form. This may lower the cost of feed and reduce the water pollution.

Acknowledgements

The authors are grateful for financial support by grant No. 39670572 from the National Natural Science Foundation of China (NNSFC). We also thank Mr. Fulong Liu of Shandong Commodity Inspection Bureau for assistance in elemental analyses.

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