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Dietary selenium requirement and its toxicity in juvenile abalone *Haliotis discus hannai* Ino

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ABSTRACT

A study was conducted to evaluate the effects of dietary selenium (Se) on survival, growth performances and immune responses of juvenile abalone *Haliotis discus hannai*. Moreover, the toxicity of high dose of dietary selenium was also analyzed. Six semi-purified diets containing graded levels of sodium selenite (0.15, 0.53, 0.88, 1.55, 2.63 and 9.16 mg Se/kg diet) were randomly fed to juvenile abalone (initial weight: 0.68 ± 0.00 g) in triplicate groups for 24 weeks in a flow-through system. Results showed that the weight gain ratio (WGR, %) of abalone fed 1.55 mg/kg of dietary selenium was significantly ($P<0.05$) higher than that with 9.16 mg/kg of dietary selenium. However, there were no significant ($P>0.05$) differences when compared to the other treatments. Selenium concentration in the soft body and the activity of glutathione peroxidase in serum were significantly ($P<0.05$) increased with dietary selenium. Significant higher activities of phenoloxidase (PO) and lysozyme in serum were observed in the treatment with 0.88 mg Se/kg diet, compared with those in 9.16 mg Se/kg diet. However, the protein concentration in serum maintained relatively constant regardless of dietary treatments. The dietary selenium requirement of juvenile abalone was estimated to be 1.408 mg/kg, using second-order polynomial regression analysis. The toxicity of high level of dietary selenium (9.16 mg/kg) to juvenile abalone was characterized by depressed growth performances and decreased activities of PO and lysozyme in serum.

1. Introduction

Selenium (Se) is both an essential and toxic trace element with a narrow margin of tolerance in biological systems (Maier and Knight, 1994). It has been found to be an integral component of glutathione peroxidase (GPx) (Rotruck et al., 1973), which is the principle factor in the protective mechanism against oxidative cellular injury (Dröge, 2002). In aquatic animals, selenium requirement has been established in several fish including rainbow trout (*Salmo Gairdnere*) (0.15–0.38 mg/kg diet) (Hilton et al., 1980), channel catfish *(Ictalurus punctatus)* (0.25 mg/kg diet) (Gatlin and Wilson, 1984) and grouper (*Epinephelus malabaricus*) (0.7 mg/kg diet) (Lin and Shiau, 2005). High doses of dietary selenium in the form of sodium selenite cause toxicity to fish and the dietary threshold can be as low as 3–5 mg/kg (Hamilton, 2004; Hilton et al., 1980). However, there is no toxicity of dietary selenomethionine up to 10 mg/kg to cutthroat trout (*Oncorhynchus clarki bouvieri*) (Hardy et al., 2010).

Abalone (*Haliotis discus hannai*) is one of the large algivorous marine gastropods, and commercially the most important species in aquaculture. However, only dietary requirements of iron, zinc and copper have been studied (Mai and Tan, 2000; Tan and Mai, 2001; Wang et al., 2009). There is no published data available on the toxicity of high doses of dietary minerals in abalone, even in mollusks. Also, limited information is available on the effect of dietary minerals on immunity and anti-oxidant system in this species (Wan et al., 2004; Wu et al., 2010). In addition to GPx, phenoloxidase (PO) is known to be a defense enzyme, and serves as a non-self recognition system in host defense reactions. Phenoloxidase activity is believed to be a sensitive indicator to reflect the immune status of invertebrates (Barracco et al., 1999; Cheng et al., 2004). Lysozyme originating from hemocytes could contribute to extracellular destruction of "invaders" (Cheng and Rodrick, 1975) and its activity is one of popular evaluative factors in the study of mollusk defense mechanisms (Bachère et al., 1995). So, GPx, PO and lysozyme were employed in this study. Hence, an experiment was conducted: (1) to estimate the dietary selenium requirement of juvenile abalone based on growth performances and immune responses, and (2) to provide a preliminary understanding on the effect of high dose of dietary selenium to abalone.

2. Materials and methods

2.1. Experimental diets and design

The formulation and proximate composition of basal diet are showed in Table 1. It was formulated with purified ingredients to

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Table 1
Ingredients and proximate composition of the basal diet.

	Percents in diet (%, dry weight)
Ingredients	
Casein	25.0
Gelatin	6.0
Dextrin	33.5
CM-cellulose	5.0
Sodium alginate	20.0
Vitamin mix [a]	2.0
Se-free mineral mix [b]	4.5
Choline chloride	0.5
SO/MFO [c]	3.5
Proximate analysis (n = 3)	
Crude protein (%)	30.8
Crude lipid (%)	3.7
Crude ash (%)	11.2
Selenium (mg/kg)	0.15

[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 B12, 0.18 mg; ascorbic acid, 4000 mg; vitamin E, 450 mg; menadione, 80 mg; retinal acetate, 100,000 IU; cholecalciferol, 2000 IU.
[b] Se-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; $CuSO_4 \cdot 5H_2O$, 12.4 mg; $CoCl_2 \cdot 6H_2O$, 0.4 mg; KIO_3, 1.2 mg.
[c] Soybean oil:menhaden fish oil = 1:1.

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 growth (Mai et al., 1995a,b). The composition of mineral premix was modified according to Tan and Mai (2001) without selenium supplementation. The composition of vitamin premix was similar to those used by Tan and Mai (2001).

The supplementation levels of selenium in diets of abalone were chosen according to dietary selenium requirements of finfish (0.15–0.7 mg/kg) (Gatlin and Wilson, 1984; Hilton et al., 1980; Lin and Shiau, 2005). The diet without selenium supplementation was considered as the deficient group (named as basal diet). High dose group was set at 9.6 mg Se/kg diet according to Hilton et al. (1980), Gatlin and Wilson (1984) and Tashjian et al. (2006), in which the threshold of dietary selenium toxicity for fish was between 10 and 20 mg/kg. Hence, the basal diet was supplemented with 0, 0.3, 0.6, 1.2, 2.4 and 9.6 mg Se/kg dry diet from $Na_2SeO_3 \cdot 5H_2O$ (Analytical Reagent, Shanghai Chemical Co., Shanghai, China). The final selenium concentrations in the experimental diets were 0.15, 0.53, 0.88, 1.55, 2.63, 9.16 mg/kg, respectively, as determined by hydride generation atomic absorption spectrophotometer (HG-AAS) (Lin and Shiau, 2005). 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. Leaching

Abalone are slow feeders. In order to determine the leaching of dietary selenium during feeding, a leaching test was carried out according to the method used by Tan and Mai (2001). Briefly, pre-weighted diet (10 g) was placed onto 100-μm-mesh screen and settled to the bottom of experimental system without abalone. At the end of 2 h (the digestive tracts of most of abalone were full of food within 2 h after feeding observed in this study), the remaining diet was removed from the system and dried overnight at 60 °C in an oven. Dried diet was analyzed for total selenium with HG-AAS (Lin and Shiau, 2005).

The results showed that dietary selenium in 0.15, 0.53 and 0.88 mg/kg treatments increased to 0.22, 0.59 and 0.98 mg/kg. However, in 1.55, 2.63 and 9.16 mg/kg treatments, dietary selenium decreased to 1.41, 2.55 and 7.40 mg/kg. The reason could be that selenium in rearing water was absorbed into the diets, or that leaching of other ingredients which led to an increase in selenium content in the diet. Although the last three diets experienced the procedure, the absorbed selenium did not balance the leaching of dietary selenium. So selenium contents in these diets decreased after 2 h of exposure to water. The remaining selenium contents in the last three diets were accounted for approximately 90.9%, 96.8% and 80.8% of the initial levels, respectively.

2.3. Animal rearing

Juvenile abalone used in this experiment were obtained from a spawning at Laoshan Fisheries Co., Shandong, China. Prior to the initiation of experiment, abalone were acclimated to laboratory conditions and fed the basal diet (Table 1) for 2 weeks. During the conditioning, 20 abalone were dissected at each time point (1 h, 1.5 h, 2 h and 3 h) after feeding to check for food in the digestive tracts. It was found that the digestive tracts of most of abalone were full of food within 2 h after feeding.

After being measured and weighed, abalone were randomly assigned to a flow-through system using a completely randomized design with 6 triplicate treatments, and each replicate at a density of 45 abalone (initial body weight: 0.68 ± 0.00 g). Each diet was fed to abalone at satiation once daily (17:00) for 24 weeks. Every morning, feces and excess feeds were removed to maintain water quality. During the experimental period, the water temperature ranged from 10 to 20 °C, salinity from 28 to 31‰ and pH from 7.8 to 8.1. Dissolved oxygen was more than 7.65 mg/l. The selenium concentration in the rearing water was 1.46 μg/l.

2.4. Sample collection

At the end of the feeding trial, animals were fasted for 3 days. All the abalone were removed from the tanks, weighed (each replicate was weighted as a group) and counted. Eight individuals from each replicate were sampled randomly for blood collection by cutting the

Table 2
Growth performances of juvenile abalone *H. discus hannai* fed different diets for 24 weeks (mean ± S.E., n = 3).

Analyzed dietary Se level (mg/kg)	Initial weight (g)	Final weight (g)	WGR[1] (%)	Survival (%)
0.15	0.68 ± 0.01	3.02 ± 0.15^{ab}	346.6 ± 27.02^{ab}	100.0 ± 0.00
0.53	0.68 ± 0.00	3.20 ± 0.23^{ab}	371.0 ± 33.83^{ab}	99.3 ± 0.74
0.88	0.68 ± 0.00	3.25 ± 0.08^{a}	380.7 ± 11.75^{ab}	100.0 ± 0.00
1.55	0.67 ± 0.00	3.47 ± 0.18^{a}	412.3 ± 28.76^{a}	99.3 ± 0.74
2.63	0.67 ± 0.00	2.98 ± 0.22^{ab}	342.9 ± 34.04^{ab}	100.0 ± 0.00
9.16	0.68 ± 0.00	2.44 ± 0.82^{b}	262.2 ± 10.73^{b}	99.3 ± 0.74

Means in the same column sharing a common superscript letter were not significantly different ($P > 0.05$) as determined by Tukey's test.
[1] WGR: weight gain ratio = [(final weight − initial weight) / initial weight] × 100.

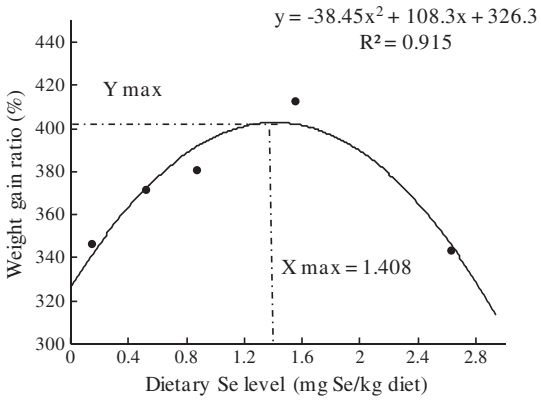

Fig. 1. The second-order polynomial regression analysis of the weight gain ratio (WGR) and dietary selenium indicates that the dietary selenium requirement of abalone is estimated to be 1.408 mg Se/kg diet. Each point represents the mean of three aquaria in a treatment. Error bars are the S.E.

blood sinus in the adductor muscle with a scalpel. Blood samples were centrifuged immediately at 3000×*g* for 10 min at 4 °C and resulting serum samples were stored at −70 °C for subsequent analyses. The soft body of the remaining abalone in each replicate were separated from the shell and collected for the selenium concentration and proximate analyses.

2.5. Sample analysis

Growth was expressed as the weight gain ratio (WGR, %). It was calculated as:

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

where W_t and W_i were the final and initial mean weight (g), respectively.

The selenium analyses in the soft body were conducted according to the method of Lin and Shiau (2005). The ground samples were digested in perchloric acid at a ratio of 1:20 (w/v), followed by the reduction of Se from the +6 to +4 state with concentrated HCl. Then the digested solution was appropriately diluted with 1% HCl within the detectable range of HG-AAS. Elemental concentrations of the samples were expressed on a dry-weight basis.

Proximate analyses of diets and animal tissues were conducted using standard procedures (Association of Official Analytical Chemists, AOAC, 1995). The protein concentration in serum was measured spectrophotometrically according to the method of Bradford (1976) using bovine serum albumin as a standard. Glutathione peroxidase activity in serum was measured according to Wan et al. (2004). Phenoloxidase and lysozyme activities in serum were measured according to Chen et al. (2005).

2.6. Statistical analysis

Data were presented as means ± standard error and subjected to one-way analysis of variance (ANOVA) after being checked for any violations of the ANOVA model. When overall differences were significant at less than 5% level, Tukey's test was used to compare the means. Statistical analysis was performed using the EXCEL 2000 and SPSS 11.5 for windows.

3. Results

3.1. Growth and survival

Survival (99.3–100.0%) was not significantly affected by dietary selenium (Table 2). Weight gain ratio (%) was increased with increasing dietary selenium from 0.15 to 1.55 mg Se/kg diet, and then significantly decreased to the lowest value at 9.16 mg Se/kg diet (Table 2). Based on the WGR, the dietary selenium requirement of juvenile abalone was estimated to be 1.408 mg/kg (Fig. 1), using second-order polynomial regression analysis.

3.2. Body composition analysis

Selenium concentration in the soft body significantly increased with dietary selenium (Table 3). The moisture (78.15–79.28%), protein (13.65–14.54%), lipid (1.14–1.26%) and ash (2.35–2.45%) contents in the soft body were not significantly affected by dietary selenium (Table 3).

3.3. Activities of GPx, PO, lysozyme and protein concentration in serum

The GPx activity in serum significantly increased with dietary selenium, and the highest value was found to be 90.47 ± 3.81 in the treatment with 9.16 mg/kg of dietary selenium (Table 4). Activities of PO and lysozyme in serum increased with dietary selenium from 0.15 to 0.88 mg/kg, and then decreased to the lowest values when dietary selenium was as high as 2.63 mg/kg (Table 4). However, the protein content (21.84–30.08 mg/ml) in serum maintained relatively constant regardless of dietary selenium concentrations (Table 4).

4. Discussion

Hilton et al. (1982) reported that rainbow trout could absorb waterborne selenium despite its low concentration (0.4–2.5 μg Se/l). In the present study, water borne selenium (1.46 μg Se/l) could not satisfy the growth requirement for juvenile abalone, and the growth rates increased with dietary selenium to the highest value when dietary selenium was up to 1.55 mg Se/kg diet. This study indicated that the selenium requirement for juvenile abalone was 1.408 mg/kg based on WGR. The requirement was higher than those reported in fish (0.15–0.7 mg Se/kg diet) based on growth data and GPx activities (Gatlin and Wilson, 1984; Hilton et al., 1980; Lin and Shiau, 2005).

Table 3
Soft body composition of juvenile abalone *H. discus hannai* fed different diets for 24 weeks (mean ± S.E., n = 3).

Analyzed dietary Se level (mg/kg)	Selenium (mg/kg)	Moisture content (%)	Crude protein (%)	Crude lipid (%)	Ash (%)
0.15	0.29 ± 0.01^{e}	78.52 ± 0.55	13.99 ± 0.40	1.24 ± 0.09	2.37 ± 0.03
0.53	0.40 ± 0.02^{de}	78.58 ± 0.54	14.32 ± 0.39	1.26 ± 0.11	2.39 ± 0.03
0.88	0.49 ± 0.01^{cd}	78.39 ± 0.79	14.40 ± 0.50	1.18 ± 0.14	2.35 ± 0.01
1.55	0.59 ± 0.01^{bc}	78.15 ± 0.21	14.54 ± 0.04	1.14 ± 0.05	2.40 ± 0.02
2.63	0.68 ± 0.03^{b}	79.28 ± 0.22	13.65 ± 0.13	1.17 ± 0.07	2.45 ± 0.02
9.16	0.81 ± 0.05^{a}	78.94 ± 0.46	13.77 ± 0.29	1.25 ± 0.07	2.37 ± 0.03

Values of the samples are expressed on a wet-weight basis.
Means in the same column sharing a common superscript letter were not significantly different ($P > 0.05$) as determined by Tukey's test.

Table 4
Enzyme activities and protein concentrations in serum of juvenile abalone *H. discus hannai* fed different diets for 24 weeks (mean ± S.E., n = 3).

Analyzed dietary Se level (mg/kg)	GPx (μmol GSH/ml/min) [1]	PO (U)[2]	Lysozyme (U)[3]	Protein (mg/ml)
0.15	29.29 ± 2.77^{c}	2.53 ± 0.06^{ab}	57.78 ± 2.22^{ab}	24.99 ± 2.27
0.53	33.33 ± 2.68^{c}	2.63 ± 0.27^{ab}	62.22 ± 4.01^{ab}	26.88 ± 3.73
0.88	48.87 ± 3.69^{b}	2.78 ± 0.18^{a}	70.56 ± 2.42^{a}	26.15 ± 1.43
1.55	57.14 ± 2.86^{b}	2.29 ± 0.20^{abc}	62.22 ± 2.22^{ab}	30.08 ± 2.02
2.63	80.48 ± 3.43^{a}	1.59 ± 0.07^{c}	48.89 ± 5.88^{b}	27.11 ± 1.41
9.16	90.47 ± 3.81^{a}	1.96 ± 0.04^{bc}	51.11 ± 4.44^{b}	21.84 ± 1.68

Means in the same column sharing a common superscript letter were not significantly different ($P > 0.05$) as determined by Tukey's test.
[1] GPx and GSH are glutathione peroxidase and glutathione, respectively.
[2] PO is phenoloxidase, One unit of PO activity is defined as the amount of enzyme causing an increase in absorbance of 0.001/min/ml serum.
[3] One unit of activity is defined as the amount of enzyme causing a decrease in absorbance of 0.001/min/ml serum.

Considering that abalone is a slow feeder, and some dietary selenium was leaching into water within 2 h, the dietary selenium requirement for abalone could be overestimated. However, there may be species-specific differences in dietary selenium requirement resulting from the different responses of growth performances to graded dietary selenium. In Nile tilapia (Kim et al., 2003), weight gain, feed efficiency ration and survival were not significantly affected by dietary selenium (from 0.2 to 0.5 mg/kg). However, in channel catfish (Gatlin et al., 1986) and Atlantic salmon (Poston et al., 1976), the growth performance (e.g. weight gain) was responsive to dietary selenium doses. On the other hand, it is generally accepted that organic selenium (e.g. selenomethionine) is more readily available than inorganic selenium (e.g. sodium selenite) to fish (Bell and Cowey, 1989; Jaramillo et al., 2009; Wang and Lovell, 1997). Correspondingly, dietary selenium requirements were lower when using organic selenium. Therefore, the requirement of dietary selenium for abalone may be further reduced to a certain extent when organic selenium is supplemented. Further study is needed to determine this.

It is known that one of the main functions of selenium is as an integral component of GPx (Rotruck et al., 1973), which is the principle factor in the protective mechanism against oxidative cellular injury (Dröge, 2002). In this study, selenium accumulation in soft body and the activity of GPx in serum were significantly increased with dietary selenium. Similar results in tissue selenium concentrations and serum GPx activities in response to the dietary selenium levels have been reported in rainbow trout (Hilton et al., 1980), channel catfish (Gatlin and Wilson, 1984) and grouper (Lin and Shiau, 2005). Wan et al. (2004) also reported in this abalone that the GPx activity in serum increased as dietary selenium increased from 0 to 1.5 mg/kg. The similar result in this abalone species was further confirmed by Wu et al. (2010), in which the transcriptions of GPx gene in haemocytes significantly increased with dietary selenium. These data suggested that dietary selenium could affect the serum GPx activities and help to enhance the function of this enzyme. However, in the present study, activities of phenoloxidase and lysozyme reached the highest values in abalone fed the diet with 0.88 mg/kg selenium and significantly decreased when dietary selenium increased to 9.16 mg/kg. The results showed that optimal dietary selenium was necessary to maintain the function of these immune related enzymes. However, there is little information on the GPx, PO and lysozyme system in non-arthropod invertebrates. More studies are needed to clarify the role of selenium in the immune system of mollusks.

Selenium is a potent toxicant to aquatic vertebrates at relatively small quantities. In the present study, significant decreased growth performances and depressed serum PO and lysozyme activities in the 9.16 mg Se/kg diet treatments suggested that high dietary selenium was toxic to juvenile abalone, although the survival was relatively high in all dietary treatments (99.3–100.0%). Hilton et al. (1980) have reported that dietary selenium from sodium selenite in excess of 3 mg/kg could be toxic to rainbow trout if maintained over long periods of time (over 20 weeks). Dietary selenium toxicity symptoms occurred at 13 and 15 mg Se/kg diet in rainbow trout and channel catfish, respectively, when sodium selenite was used as the dietary selenium source. The symptoms included the reduced growth rate, poor feed efficiency and high mortality (Gatlin and Wilson, 1984; Hilton et al., 1980). In the present study, as a slow feeder species, the 24-week experiment period may be not long enough for high dose of dietary selenium (9.16 mg/kg) to cause high mortality, although abalone were in the sublethal status with the poor growth performances and enzyme (PO and lysozyme) activities. Spallholz and Hoffman (2002) concluded in aquatic bird that one of the important mechanisms of selenium toxicity appears to involve the formation of CH_3Se^- that either enters a redox cycle and generates superoxide and oxidative stress, or forms free radicals that bind to and inhibit important enzymes and proteins. This could be further confirmed by the significant decreased enzyme activities of PO and lysozyme in serum of abalone exposed to 9.16 mg Se/kg diet in this study and the significant down-regulated expression level of GPx mRNA in hepatopancreas of this abalone species when exposed to dietary selenium of 48.7 mg/kg (Wu et al., 2010). So far, there is no information available on the mechanism of selenium toxicity in mollusks. In this respect, it is interesting to focus further research on it.

In summary, dietary selenium significantly affected the growth performances and immune status of juvenile abalone. The second-order polynomial regression analysis of WGR indicated that the dietary selenium requirement of juvenile abalone was estimated to be 1.408 mg/kg. The toxicity of high dose of dietary selenium (9.16 mg/kg) to juvenile abalone was characterized by depressed growth performances and decreased activities of PO and lysozyme in serum.

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