



Dose-dependent protective effects of dietary selenium on abalone *Haliotis discus hannai* Ino against the toxicity of waterborne copper

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Abstract

This study was designed to assess the protective effects of dietary selenium (Se) on abalone *Haliotis discus hannai* Ino against the toxicity of waterborne copper (Cu). A 60-day feeding trial was conducted in a static water system for abalone (initial weight: 3.17 ± 0.01 g) exposed to 0.02 mg L^{-1} of waterborne Cu. The animals were fed one of the three experimental diets with 0.10, 1.31 and 4.20 mg kg^{-1} of Se from $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$ respectively. Results showed that the abalone fed 1.31 mg kg^{-1} of dietary Se had the lowest Cu concentration in shell, muscle, mantle, gill, hepatopancreas and serum. Meanwhile, the significant lowest contents of malondialdehyde and protein carbonyl in hepatopancreas were also found in the treatment with 1.31 mg kg^{-1} of dietary Se ($P < 0.05$). In addition, this treatment had significant higher glutathione content and thioredoxin reductase activity in hepatopancreas ($P < 0.05$). However, the activity of Se-dependent glutathione peroxidase (Se-GPx) was significantly decreased in the treatment with 4.20 mg kg^{-1} of dietary Se ($P < 0.05$). In this treatment, the protein carbonyl content in hepatopancreas was significantly higher than that in the group with 1.31 mg kg^{-1} of dietary Se ($P < 0.05$). In conclusion, in terms of anti-oxidation and Cu accumulation, the protective effects of dietary Se on abalone against waterborne Cu were dose-dependent. The 1.31 mg kg^{-1} of dietary Se had this effect, but not 4.20 mg kg^{-1} of dietary Se. Moreover, the latter increased the oxidative stress in abalone exposed to the waterborne Cu.

Keywords: *Haliotis discus hannai*, selenium, copper, anti-oxidation, toxicity

Introduction

Heavy metals represent a significant ecological and public health concern due to their toxicity and bioaccumulation. It is well-known that copper (Cu) is an essential trace metal for all biota and it is present in all natural waters and sediments (Linder 2001). But at a relatively high concentration in the environment, Cu can be toxic to aquatic organisms. In our previous study, it was found that abalone *Haliotis discus hannai* Ino was more sensitive to waterborne Cu than to Cd. Furthermore, 0.04 mg L^{-1} of Cu in seawater resulted in the death of abalone (Lei, Zhang, Xu & Mai 2015). Elevated Cu concentrations in water can induce the overproduction of reactive oxygen species (ROS) (Upadhyay & Panda 2010). Oxidative stress occurs when there is excessive ROS production and/or a low anti-oxidative defence, which can damage cell membrane lipids, proteins and nucleic acids (Bagchi, Bagchi, Hassoun & Stohs 1996; Srivastava, Mishra, Tripathi, Dwivedi & Gupta 2006). Compared with that in the natural seawater with $4.50 \mu\text{g L}^{-1}$ of Cu, abalone *H. discus hannai* exposed to waterborne Cu ranging from 0.02 to 0.08 mg L^{-1} had more severe lipid peroxidation (LPO; Lei *et al.* 2015).

Selenium (Se) is regarded as a natural antioxidant to heavy metal toxicity (Frost 1981). It is an important component of anti-oxidative enzymes, such as thioredoxin reductase (TrxR) and glutathione peroxidase (GPx). These enzymes together

with superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), thioredoxin peroxidase (TrxP) and the reduced glutathione (GSH) can protect cells against damage caused by free radicals and lipoperoxides induced by heavy metals (Finkel & Holbrook 2000). Previous studies have described important beneficial properties of Se as an antioxidant (Machado Mda, Villela, Moura, Rosa, Salvador, Lopes, Braga, Roesler, Saffi & Henriques 2009), and a protector against cadmium (Frisk, Yaqob & Lindh 2002) and arsenic (Roussyn, Briviba, Masumoto & Sies 1996). Trevisan, Mello, Fisher, Schuwerack, Dafre and Moody (2011) demonstrated that the pre-exposure to waterborne Se largely prevented oxidation of protein thiols and DNA damage in blue mussel *Mytilus edulis* induced by Cu.

Abalone *H. discus hannai* is the most commercially important marine gastropod cultured in China. In the recent years, abalone culture has suffered serious problems of mortality from disease outbreaks, including heavy metal stress (Jing, Lia, Xie & Zhang 2007). Feed may be a carrier for the substance that can potentially improve the anti-stress capacity of abalone. The aim of this study was to investigate the effects of dietary Se in terms of anti-oxidation and heavy metal accumulation, on abalone *H. discus hannai* against the toxicity of waterborne Cu.

Materials and methods

Abalone

Three hundred and sixty healthy juvenile abalone *H. discus hannai* (initial body weight: 3.17 ± 0.01 g) were collected from a spawning at Laoshan Fisheries, Qingdao, China. Prior to the feeding trial, animals were acclimated to the laboratory conditions for 2 weeks. They were fed fresh kelp once daily at 18:00 hours. Every morning, faeces or uneaten kelp was removed to maintain the water quality.

Experimental design and sample collection

The feeding trial was conducted in tanks (100 L) in a static system for 60 days. A stock solution was prepared by dissolving $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in natural seawater to achieve a final Cu concentration of $1000 \mu\text{g L}^{-1}$. Then, this solution was diluted in the natural seawater to achieve the designed

concentration ($0.02 \text{ mg Cu L}^{-1}$ seawater) in a large aquarium (350 L). The prepared seawater was pumped to each experimental tank. The analysed concentration of waterborne Cu was $0.018 \pm 0.001 \text{ mg L}^{-1}$. According to Lei *et al.* (2015), after being exposed to the waterborne Cu ($0.02, 0.04, 0.06$ and 0.08 mg L^{-1} , respectively) for 28 days, only the abalone with 0.02 mg L^{-1} of waterborne Cu survived. The $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$ was used as the dietary Se source to design three supplemented levels of dietary Se. They were 0, 1.5 and 4.5 mg kg^{-1} respectively. The analysed concentrations of dietary Se were 0.10, 1.31 and 4.20 mg kg^{-1} respectively. Selenium concentrations in the seawater were $0.46 \mu\text{g L}^{-1}$.

The basal diet formulation was based on that of Mai, Zhang, Tan and He (2003) with some modifications. The diet was formulated with purified ingredients (Table 1) to provide 29.41% crude protein from casein and gelatin, and 3.26% crude lipid from soybean oil and menhaden fish oil (1:1), which would be considered to be sufficient to maintain

Table 1 Ingredient and proximate composition of the basal diet

Ingredient	Contents (%)
Casein*	25.00
Gelatin†	6.00
Dextrin‡	33.50
Carboxymethyl cellulose‡	5.00
Sodium alginate†	20.00
Vitamin mix‡	2.00
Mineral mix§	4.50
Choline chloride†	0.50
SO/MFO¶	3.50
Proximate analysis (dry weight %)	
Crude protein	29.41
Crude lipid	3.26
Crude ash	10.01

*Sigma Chemical, St Louis, MO, USA.

†Shanghai Chemical, Shanghai, China.

‡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; ascorbic acid, 4000 mg; B₁₂, 0.18 mg; vitamin E, 450 mg; menadione, 80 mg; retinol acetate, 100 000 IU; cholecalciferol, 2000 IU.

§Mineral mix, each 1000 g of diet contained: NaCl, 0.4 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 6.0 g; $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 10.0 g; KH_2PO_4 , 20.0 g; $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$, 8.0 g; Fe-citrate, 1.0 g; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 141.2 mg; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 64.8 mg; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 12.4 mg; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.4 mg; KIO_3 , 1.2 mg.

¶Soybean oil and menhaden fish oil (1:1).

optimal growth for *H. discus hannai* (Mai, Mercer & Donlon 1995a,b). Procedures for diet preparation and storage were as previously described (Zhang, Mai, Xu, Tan, Ai, Liufu, Ma & Wang 2007).

After measuring the body weight, abalone were assigned to a static system using a completely randomized design with three triplicated treatments. Half of the water was exchanged with fresh seawater twice daily. Each replicate (tank) consisted of 40 abalone. Diets were hand-fed to abalone to satiation once daily at 18:00 hours. Every morning, faeces and uneaten feed were removed to maintain the water quality. During the 60-day feeding trial, the water temperature was 17–20°C, salinity 22–27 g L⁻¹, pH 7.4–7.9 and dissolved oxygen was not below 6 mg L⁻¹. The photoperiod regime during the feeding trial was 12-h light:12-h dark.

At the end of the feeding trial, all abalone were removed from the tanks, then weighed (each replicate was weighted as a group) and counted. The shell, muscle, mantle, gill and hepatopancreas were collected. The muscle, mantle, gill and hepatopancreas were immediately washed with cold saline (0.86% NaCl) and frozen in liquid nitrogen. The haemolymph from abalone was taken by use of syringes and needles from the adductor muscles. Serum was collected and immediately frozen in liquid nitrogen. Serum samples from all abalone of each replicate were pooled for the analysis. The hepatopancreas was stored at –80°C. The shell, muscle, mantle, gill and serum were stored at –20°C. Growth was expressed as specific growth rate (SGR, % per day⁻¹). The calculation formula is as follows:

$$\text{SGR}(\%/ \text{day}) = 100 * (\ln(Wt) - \ln(Wi)) / t$$

where Wt and Wi are the final and initial body weight (g) respectively. And *t* is the exposure time (days).

Proximate analyses of crude protein, crude lipid and crude ash in diets were conducted following the standard procedures (AOAC, 1995).

Selenium and copper determination

The water samples were kept in dark and stored at –20°C until further analysis. The shell, muscle, mantle, gill and hepatopancreas samples were lyophilized for 12 h. Powdered samples (about 100 mg) or 500 µL serum were acidified with 10 mL perchloric acid. The Se concentrations

in water samples were determined by flame atomic absorption spectrophotometry (SOLAAR M6; Thermo, Waltham, MA, USA). The Cu concentrations in seawater and abalone tissues were determined by an inductively coupled plasma-atomic emission spectrophotometer (ICP-OES; VISTA-MPX; VARIAN, Palo Alto, CA, USA).

Anti-oxidative enzymes activities

Activity of CAT was determined using a spectrophotometric assay of hydrogen peroxide (Goth 1991). One unit of CAT activity was defined as the degradation of 1 µmol H₂O₂ per second per mg tissue protein.

Activity of the SOD was determined through the inhibition of nitrobluetetrazolium reduction by O₂⁻ generated by the xanthine/xanthine oxidase system (Huang, Zhou & Zhang 2006). One SOD activity unit was defined as the enzyme amount causing 50% inhibition in 1 mL reaction solution per mg tissue protein. The result was expressed as U mg⁻¹ protein.

Activity of the Se-dependent glutathione peroxidase (Se-GPx) was assayed spectrophotometrically by measuring the decrease in the enzymatic reaction of glutathione at 412 nm (Li, Zhang & Liu 2005). One unit of Se-GPx activity was defined as the decrease in the amount of 1 µmol L⁻¹ glutathione in the enzymatic reaction system of 1 mg protein min⁻¹. The activity of Se-GPx was expressed as U mg⁻¹ protein.

Activity of GST was determined using the conjugation of 1-chloro-2,4-dinitrobenzene with reduced glutathione (Habig, Pabst & Jakoby 1974). One unit of GST activity was defined as the decrease in the amount of 1 µmol L⁻¹ glutathione in the enzymatic reaction system of 1 mg protein min⁻¹.

Activity of the TrxR was performed spectrophotometrically, which involved the NADPH-dependent reduction in the disulphide bond in 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) by TrxR (Oblong, Gasdaska, Sherrill & Powis 1993). The activities were expressed as micromoles of DTNB reduced to TNB g⁻¹ of the soluble lens protein.

Activity of the TrxP was measured using the commercial detection kit (Nanjing Jiancheng Bio-engineering Institute, Nanjing, China).

Protein concentration in all samples was measured spectrophotometrically by the method of Bradford (1976) with bovine serum albumin as standard.

Glutathione contents

The reduced GSH content in the hepatopancreas was determined as described by Anderson (1985). Glutathione was quantified using a standard curve of known concentrations of GSH.

Lipid peroxidation assay

Level of malondialdehyde (MDA) in hepatopancreas was measured using the thiobarbituric acid fluorometric assay with 1,1,3,3-tetraethoxypropane as a standard (Şahin, Sağdıç, Elmas, Akpınar, Derin, Aslan, Agar, Alicigüzel & Yargıçoğlu 2007). Levels of MDA were determined fluorometrically with excitation and emission wavelengths of 532 and 547 nm respectively.

DNA alkaline unwinding assay

DNA strand-breaks were determined according to DNA alkaline unwinding assay adapted from Ching, Siu, Lam, Xu, Zhang, Richardson and Wu (2001). In the assay, the rate of transition of double-stranded DNA (dsDNA) to single-stranded DNA (ssDNA) under pre-defined alkaline denaturing conditions was proportional to the number of breaks in the phosphodiester backbone, and thus was used as a measure of DNA integrity (Daniel, Haas & Santi 1985). The DNA sample was diluted and separated into three equal portions for fluorescence determination of dsDNA, ssDNA and partially unwound DNA (auDNA). The ratio between double-stranded DNA and total DNA (F -value) was determined as follows: F -value = (auDNA – ssDNA)/(dsDNA – ssDNA).

Protein degradation assay

Protein carbonyl content was measured by forming labelled protein hydrazone derivatives, using

2,4-dinitro-phenylhydrazide, and then quantified spectrophotometrically following the method of Mecocci, Fanò, Fulle, MacGarvey, Shinobu, Polidori, Cherubini, Vecchiet, Senin and Beal (1999). The carbonyl content was calculated as nmol mg⁻¹ protein.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 (SPSS, Chicago, IL, USA) and Microsoft Excel 2003. Data were analysed by one-way ANOVA. When significant differences ($P < 0.05$) were found, means were compared using the Tukey's test. All the data were presented as means \pm SE.

Results

There were no significant differences in survival rate (88.33–98.33%) among treatments ($P = 0.101$; Table 2). No significant difference was found in SGR among the three treatments ($P = 0.269$). The highest SGR ($0.66 \pm 0.08\%$ per day) was found in the treatment with 4.20 mg kg⁻¹ of dietary Se.

As showed in Table 3, dietary Se significantly influenced the Cu concentrations in serum ($P = 0.01$) and hepatopancreas ($P = 0.01$). In the hepatopancreas, the Cu concentration in the treatment with 1.31 mg kg⁻¹ of dietary Se was significantly lower than those in the other two treatments ($P < 0.05$). In serum, the treatments with 1.31 and 4.20 mg kg⁻¹ of dietary Se had significantly lower Cu concentrations than the control ($P < 0.05$). No significant difference was found in Cu concentration in shell ($P = 0.573$), muscle ($P = 0.219$), mantle ($P = 0.685$) and gill ($P = 0.523$) among the treatments.

The activities of SOD ($P = 0.936$), CAT ($P = 0.883$), GST ($P = 0.290$) and TrxP ($P = 0.434$) were not significantly influenced by dietary

Dietary selenium (mg kg ⁻¹)	Initial weight (g)	Final weight (g)	Specific growth rate (% per day ⁻¹)	Survival (%)
0.10	3.15 \pm 0.00	4.25 \pm 0.22	0.50 \pm 0.09	98.33 \pm 0.83
1.31	3.17 \pm 0.01	4.58 \pm 0.07	0.62 \pm 0.03	96.67 \pm 1.67
4.20	3.17 \pm 0.02	4.72 \pm 0.20	0.66 \pm 0.08	88.33 \pm 4.64
One-way ANOVA				
P -value	0.592	0.232	0.269	0.101
F -value	0.572	1.883	1.648	3.444

Table 2 Effects of dietary selenium on growth and survival of abalone *Haliotis discus hannai* Ino exposed to waterborne Cu for 60 days (mean \pm SE, $n = 3$)

Table 3 Effects of dietary selenium on Cu accumulation in tissues of abalone *Haliotis discus hannai* Ino exposed to waterborne Cu for 60 days (mean \pm SE, $n = 3$)

Dietary selenium (mg kg ⁻¹)	Serum ($\mu\text{g mL}^{-1}$)	Shell ($\mu\text{g g}^{-1}$)	Muscle ($\mu\text{g g}^{-1}$)	Mantle ($\mu\text{g g}^{-1}$)	Gill ($\mu\text{g g}^{-1}$)	Hepatopancreas ($\mu\text{g g}^{-1}$)
0.10	5.29 \pm 0.32 ^a	19.49 \pm 3.69	8.76 \pm 0.37	15.28 \pm 1.88	13.92 \pm 1.27	13.66 \pm 0.49 ^a
1.31	2.74 \pm 0.33 ^b	16.39 \pm 1.92	7.98 \pm 0.45	14.81 \pm 1.84	13.96 \pm 2.44	9.08 \pm 0.51 ^b
4.20	3.52 \pm 0.08 ^b	21.14 \pm 3.35	9.18 \pm 0.48	16.93 \pm 1.52	16.45 \pm 1.06	13.44 \pm 0.58 ^a
One-way ANOVA						
<i>P</i> -value	0.001	0.573	0.219	0.685	0.523	0.001
<i>F</i> -value	23.477	0.611	1.975	0.403	0.724	23.579

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

Table 4 Effects of dietary selenium on the anti-oxidative parameters in hepatopancreas of abalone *Haliotis discus hannai* Ino exposure to waterborne Cu for 60 days (mean \pm SE, $n = 3$)

Dietary selenium (mg kg ⁻¹)	SOD	CAT	Se-GPx	GST	GSH	TrxR	TrxP
0.10	10.77 \pm 0.38	6.42 \pm 1.53	16.58 \pm 1.02 ^a	2.59 \pm 0.33	4.15 \pm 0.06 ^b	2.20 \pm 0.06 ^b	4.86 \pm 0.38
1.31	11.04 \pm 0.81	6.78 \pm 1.80	16.39 \pm 1.09 ^a	2.31 \pm 0.15	5.13 \pm 0.21 ^a	2.58 \pm 0.05 ^a	5.10 \pm 0.39
4.20	10.79 \pm 0.44	5.63 \pm 1.61	10.51 \pm 1.67 ^b	2.06 \pm 0.10	5.52 \pm 0.07 ^a	2.36 \pm 0.02 ^b	5.52 \pm 0.23
One-way ANOVA							
<i>P</i> -value	0.936	0.883	0.026	0.290	0.001	0.004	0.434
<i>F</i> -value	0.067	0.127	7.101	1.530	27.316	16.163	0.961

SOD, total superoxide dismutase (U mg⁻¹ Prot); CAT, catalase (U mg⁻¹ Prot); GPX, glutathione peroxidase (U mg⁻¹ Prot); GST, glutathione S-transferases (U mg⁻¹ Prot); GSH, glutathione (mg g⁻¹ Prot); TrxR, thioredoxin reductase (mU L⁻¹); TrxP, thioredoxin peroxidase (U mg⁻¹ Prot).

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

Se (Table 4). The activity of SOD, CAT and TrxP in the treatment with 1.31 mg kg⁻¹ of dietary Se was higher than those in the other two treatments ($P > 0.05$).

The dietary Se contents significantly influenced the activity of Se-GPx ($P = 0.026$), TrxR ($P = 0.001$) and the content of GSH ($P = 0.004$) in the hepatopancreas. The Se-GPx activity in the treatment with 4.20 mg kg⁻¹ of dietary Se was significantly lower than those in the other two treatments ($P < 0.05$). However, Se-GPx activity in the treatment with 1.31 mg kg⁻¹ of dietary Se was not significantly different from the control. The activity of TrxR and the content of GSH in the treatment with supplemented dietary Se were significantly higher than those in the control ($P < 0.05$).

As shown in Table 5, the MDA content in the treatment with 1.31 mg kg⁻¹ of dietary Se was significantly lower than that in the control

($P < 0.05$). The treatment with 4.20 mg kg⁻¹ of dietary Se had no significant difference in MDA content compared with the control ($P > 0.05$). The protein carbonyl content in 1.31 mg kg⁻¹ dietary Se group was significantly lower than those in the other two treatments ($P < 0.05$). No significant difference in *F*-values was found among all the treatments ($P = 0.186$; Table 5).

Discussion

Copper toxicity associated with consumption of excess dietary Cu, high liver Cu concentrations and hepatocellular necrosis, is a well-recognized problem in animals (Soli 1980). In the present work, 1.31 mg kg⁻¹ of dietary Se caused a significant reduction in Cu accumulation in the hepatopancreas and serum compared with the control or the treatment, which received 4.20 mg kg⁻¹ of dietary Se (Table 2). Similarly, in the previous

Table 5 Effects of dietary selenium on the oxidative parameters in hepatopancreas of abalone *Haliotis discus hannai* Ino exposure to waterborne Cu for 60 days (mean \pm SE, $n = 3$)

Dietary selenium (mg kg ⁻¹)	MDA (nmol mg ⁻¹ Prot)	Protein carbonyl (nmol mg ⁻¹ Prot)	DNA strand-breaks (F-value)
0.10	29.94 \pm 1.05 ^a	8.67 \pm 0.23 ^a	0.38 \pm 0.08
1.31	21.79 \pm 0.95 ^b	4.35 \pm 0.32 ^b	0.26 \pm 0.02
4.20	24.41 \pm 0.91 ^{ab}	8.49 \pm 1.20 ^a	0.25 \pm 0.01
One-way ANOVA			
P-value	0.016	0.009	0.186
F-value	8.918	11.232	2.254

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

Cu-exposed study, Trevisan *et al.* (2011) found that the accumulation of Cu in gills was lower in blue mussel *M. edulis* pre-exposed to water Se (4 $\mu\text{g L}^{-1}$) for 3 days than those with no Se pre-exposure. In this study, the content of GSH in the treatment with 1.31 mg kg⁻¹ of dietary Se was significantly higher than that in the control (Table 4). It has been reported that the sulfhydryl group of cysteine moiety of GSH has a high affinity for Cu to form thermodynamically stable mercaptide complexes, which are inert and will be excreted outside by animal (Wang & Ballatori 1998). It was suggested that GSH induced by dietary Se, in this study, could play an important role in the detoxification of Cu.

The toxic effect of heavy metal appears to be related to the production of ROS (Winterbourn 1982), and the reduction in the cellular anti-oxidative capacity (Sies 1999). In our previous study, activities of anti-oxidative enzymes (e.g. SOD, GPx and GST) in the hepatopancreas of abalone *H. discus hannai* were inhibited under 0.02 mg L⁻¹ waterborne Cu exposure for 28 days (Lei *et al.* 2015). Selenium can act as an antioxidant in animals. The most important evidence of this function is the close localization of Se to the active site of many anti-oxidative enzymes, such as TrxR and GPx in the cell (Rotruer & Poue 1993; Maleki, Safavi & Doroodmand 2005). It was found in rats that administration of Se at 3 mg kg⁻¹ body weight in As-induced toxicity protected liver from LPO and from any changes in GSH and anti-oxidative enzymes activities (Combs & Combs 1984; McPherson 1994; Ji, Wang,

Cheng, Yuan, Zhao, Zhuang & Qu 2006). The possible role of Se in the enhancement of anti-oxidative processes was also found in detoxification of Cd (Newairy, El-Sharakly, Badredeen, Eweda & Sheweita 2007) or Cu (Tran, Moody, Fisher, Foulkes & Jha 2007). In the present study, activities of the anti-oxidative enzymes including SOD, CAT, TrxR and TrxP in the 1.31 mg kg⁻¹ dietary Se treatment were higher than those in the control (Table 4). It was suggested that these enzymes induced by dietary Se were involved in detoxifying the toxicity of the waterborne Cu.

Malondialdehyde is a general indicator for LPO. Chelomin and Belcheva (1991) demonstrated that Cu accumulation in hepatopancreas cells was accompanied by a significant increase in MDA concentration in scallop *Mizuhopectin yessoensis*. In this study, MDA content in the treatment with 1.31 mg kg⁻¹ of dietary Se was significantly lower than that in the control (Table 5). Orun, Ates, Selamoglu, Yazlak, Ozturk and Yilmaz (2005) also reported decreased MDA levels in the liver and other organs in rainbow trout *Oncorhynchus mykiss* exposed to 2–6 mg L⁻¹ sodium selenite for 72 h. Similar results were also found in grouper *Epinephelus malabaricus* (Lin & Shiao 2007). The above studies suggested that Se played an important role in reducing the LPO induced by Cu.

Reactive oxygen species cause a number of non-enzymatic protein modifications, including protein carbonylation, which is a widely used biomarker of oxidative stress (Shacter, Williams, Lim & Levine 1994; Je, Lee, Kim, Cho, Lee, Kim, Lee & Lee 2008). The formation of carbonyl derivatives is irreversible and increases the susceptibility of proteins to proteases (Stadtman & Oliver 1991). Kaloyianni, Dailianis, Chrisikopoulou, Zannou, Koutsogiannaki, Alamdari, Koliakos and Dimitriadis (2009) reported that the significant increase in protein carbonylation content was detected in haemolymph of mussels *M. galloprovincialis* exposed to different concentrations of metals. In this study, protein carbonylation content in the treatment with 1.31 mg kg⁻¹ of dietary Se was significantly lower than in the control (Table 5).

Copper exposure also causes an increase in the DNA damage, as observed in erythrocytes in gilt-head bream *Sparus aurata* and haemocytes in clam *Scapharca inaequivalvis* (Gabbianelli, Lupidi, Villarini & Falcioni 2003). According to Tran *et al.* (2007), Se supplementation (4 $\mu\text{g L}^{-1}$) could significantly reduce mercury-induced DNA damage in *M. edulis*.

Trevisan *et al.* (2011) also found that pre-treatment with Se largely prevented these deleterious effects of Cu on DNA damage. However, in this study, there were no significant differences in DNA damages among treatments (Table 5). It is suggested that dietary Se had no significant effects on the protection of abalone against DNA damage from waterborne Cu. Further study is needed.

Selenium is both an essential and toxic trace element with a narrow margin of tolerance in biological systems (Maier & Knight 1994). Hilton, Hodson and Slinger (1980) reported that dietary Se in excess of 3 mg kg⁻¹ could be toxic to rainbow trout. Dietary Se toxicity symptoms occurred at 15 mg kg⁻¹ Se diet in channel catfish (Gatlin & Wilson 1984). The symptoms included reduced growth rate, poor feed conversion efficiency and high mortality (Gatlin & Wilson 1984). A similar result was found in the present study. Compared with the treatment with 1.31 mg kg⁻¹ of dietary Se, higher Se diet (4.20 mg kg⁻¹) resulted in higher Cu accumulation in shell, muscle, mantle, gill and hepatopancreas (Table 2). Moreover, the decreases of LPO levels and protein carbonylation contents in the hepatopancreas were not found in the treatment with 4.2 mg kg⁻¹ of dietary Se (Table 4). It is suggested that Se is a potential toxicant to aquatic animals at relatively small quantities. Thus, potential hazard of Se should be considered carefully.

Conclusion

In terms of the anti-oxidation and metal accumulation, protective effects of dietary Se on abalone exposed to waterborne Cu depended on the dose of Se. When dietary Se content was 1.31 mg kg⁻¹, it significantly increased the GSH content and TrxR activity in hepatopancreas. It significantly decreased the Cu concentrations in serum and hepatopancreas, the contents of MDA and protein carbonyl in hepatopancreas. When dietary Se content increased to 4.20 mg kg⁻¹, however, such protection effect of dietary Se on abalone against waterborne Cu disappeared as it significantly increased the protein carbonyl content in the hepatopancreas.

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References

- Anderson M.E. (1985) Determination of glutathione and glutathione disulfide in biological samples. *Methods in Enzymology* **113**, 548–555.
- Association of Official Analytical Chemists (AOAC) (1995) *Official Methods of Analysis*, 16th edn, pp. 1–45. AOAC, Arlington, VA, USA.
- Bagchi D., Bagchi M., Hassoun E.A. & Stohs S.J. (1996) Cadmium-induced excretion of urinary lipid metabolites, DNA damage, glutathione depletion, and hepatic lipid peroxidation in Sprague-Dawley rats. *Biological Trace Element Research* **52**, 143–154.
- Bradford M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248–254.
- Chelomin V.P. & Belcheva N.N. (1991) Alterations of microsomal lipid synthesis in gill cells of bivalve mollusk *Mizuhopecten yessoensis* in response to cadmium accumulation. *Comparative Biochemistry Physiology Part C: Toxicology and pharmacology* **99**, 1–5.
- Ching E.W.K., Siu W.H.L., Lam P.K.S., Xu L., Zhang Y.Y., Richardson B.J. & Wu R.S.S. (2001) DNA adduct formation and DNA strand breaks in green-lipped mussels (*Perna viridis*) exposed to benzo[a]pyrene: dose- and time-dependent relationships. *Marine Pollution Bulletin* **42**, 603–610.
- Combs G.F. & Combs S.B. (1984) The nutritional biochemistry of selenium. *Annual Review of Nutrition* **4**, 257–280.
- Daniel F.B., Haas D.L. & Santi L. (1985) Quantitation of chemically induced DNA strand breaks in human cells via an alkaline unwinding assay. *Analytical Biochemistry* **144**, 390–402.
- Finkel T. & Holbrook N.J. (2000) Oxidants, oxidative stress and the biology of ageing. *Nature* **408**, 239–247.
- Frisk P., Yaqob A. & Lindh U. (2002) Indication of selenium protection against cadmium toxicity in cultured K-562 cells. *Science of the Total Environment* **296**, 189–197.
- Frost D.V. (1981) Selenium and vitamin E as antidotes to heavy metal toxicities. In: *Selenium in Biology and Medicine* (ed. by J.E. Spallholz), pp. 490–498. AVC Publishing, Westport, CT, USA.
- Gabbianelli R., Lupidi G., Villarini M. & Falcioni G. (2003) DNA damage induced by copper on erythrocytes of gilthead sea bream *Sparus aurata* and mollusk *Scapharca inaequivalvis*. *Archives of Environmental Contamination and Toxicology* **45**, 350–356.
- Gatlin I.I.D.M. & Wilson R.P. (1984) Dietary selenium requirement of fingerling channel catfish. *Journal of Nutrition* **114**, 627–633.
- Goth L. (1991) A simple method for determination of serum catalase activity and revision of reference range. *Clinica Chimica Acta* **196**, 143–151.

- Habig W.H., Pabst M.J. & Jakoby W.B. (1974) Glutathione S-transferases. The first step in mercapturic acid formation. *Journal of Biological Chemistry* **249**, 7130–7139.
- Hilton J.W., Hodson P.V. & Slinger S.J. (1980) The requirement and toxicity of selenium in rainbow trout (*Salmo gairdneri*). *Journal of Nutrition* **110**, 2527–2535.
- Huang X.X., Zhou H.Q. & Zhang H. (2006) The effect of *Sargassum fusiforme* polysaccharide extracts on vibriosis resistance and immune activity of the shrimp *Fenneropenaeus chinensis*. *Fish and Shellfish Immunology* **20**, 750–757.
- Je J.H., Lee T.H., Kim D.H., Cho Y.H., Lee J.H., Kim S.C., Lee J. & Lee M.G. (2008) Mitochondrial ATP synthase is a target for TNBS-induced protein carbonylation in XS-106 dendritic cells. *Proteomics* **8**, 2384–2393.
- Ji X., Wang W., Cheng J., Yuan T., Zhao Y., Zhuang H. & Qu L. (2006) Free radicals and antioxidant status in rat liver after dietary exposure of environmental mercury. *Environmental Toxicology and Pharmacology* **22**, 309–314.
- Jing G., Lia Y., Xie L. & Zhang R. (2007) Different effects of Pb^{2+} and Cu^{2+} on immune and antioxidant enzyme activities in the mantle of *Pinctada fucata*. *Environmental Toxicology and Pharmacology* **24**, 122–128.
- Kaloyianni M., Dailianis S., Chrisikopoulou E., Zannou A., Koutsogiannaki S., Alamdari D.H., Koliakos G. & Dimitriadis V.K. (2009) Oxidative effects of inorganic and organic contaminants on haemolymph of mussels. *Comparative Biochemistry Physiology Part C: Toxicology and pharmacology* **149**, 631–639.
- Lei Y., Zhang W., Xu W. & Mai K. (2015) Effects of waterborne copper and cadmium on antioxidant response, lipid peroxidation and heavy metals accumulation in abalone *Haliotis discus hannai* Ino. *Journal of Ocean University of China* **14**, 511–521.
- Li L.J., Zhang F. & Liu X.M. (2005) Oxidative stress related enzymes in response to chromium (VI) toxicity in *Oxya chinensis* (Orthoptera: Acridoidae). *Journal of Environmental Sciences* **17**, 823–826.
- Lin Y. & Shiao S. (2007) The effects of dietary selenium on the oxidative stress of grouper, *Epinephelus malabaricus*, fed high copper. *Aquaculture* **267**, 38–43.
- Linder M.C. (2001) Copper and genomic stability in mammals. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* **475**, 141–152.
- Machado Mda S., Villela I.V., Moura D.J., Rosa R.M., Salvador M., Lopes N.P., Braga A.L., Roesler R., Saffi J. & Henriques J.A. (2009) 3′3-Ditrifluoromethylidiphenyl diselenide: a new organoselenium compound with interesting antigenotoxic and antimutagenic activities. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* **673**, 133–140.
- Mai K., Mercer J.P. & Donlon J. (1995a) Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L and *Haliotis discus hannai* Ino. IV. Optimum dietary protein level for growth. *Aquaculture* **136**, 165–180.
- Mai K., Mercer J.P. & Donlon J. (1995b) Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L and *Haliotis discus hannai* Ino. III. Response of abalone to various levels of dietary lipid. *Aquaculture* **134**, 65–80.
- Mai K., Zhang W., Tan B. & He G. (2003) Effects of dietary zinc on the shell biomineralization in abalone *Haliotis discus hannai* Ino. *Journal of Experimental Marine Biology and Ecology* **283**, 51–62.
- Maier K.J. & Knight A.W. (1994) Ecotoxicology of selenium in freshwater system. In: *Reviews of Environmental Contamination and Toxicology*, Vol. 134 (ed. by G.W. Ware), pp. 31–48. Springer-Verlag, New York, USA.
- Maleki N., Safavi A. & Doroodmand M.M. (2005) Determination of selenium in water and soil by hydride generation atomic absorption spectrometry using solid reagents. *Talanta* **66**, 858–862.
- McPherson A. (1994) Selenium vitamin E and biological oxidation. In: *Recent Advances in Animal Nutrition* (ed. by D.J. Cole & P.J. Garnsworthy), pp. 3–30. Butterworth and Heinemann's, Oxford.
- Mecocci P., Fanò G., Fulle S., MacGarvey U., Shinobu L., Polidori M.C., Cherubini A., Vecchiet J., Senin U. & Beal M.F. (1999) Age dependent increases of oxidative damage to DNA, lipids, and proteins in human skeletal muscle. *Free Radical Biology and Medicine* **26**, 303–308.
- Newairy A.A., El-Sharaky A.S., Badreldeen M.M., Eweda S.M. & Sheweita S.A. (2007) The hepatoprotective effects of selenium against cadmium toxicity in rats. *Toxicology* **242**, 23–30.
- Oblong J.E., Gasdaska P.Y., Sherrill K. & Powis G. (1993) Purification of human thioredoxin reductase: properties and characterization by absorption and circular dichroism spectroscopy. *Biochemistry* **32**, 7271–7277.
- Orun I., Ates B., Selamoglu Z., Yazlak H., Ozturk E. & Yilmaz I. (2005) Effects of various sodium selenite concentrations on some biochemical and hematological parameters of rainbow trout (*Oncorhynchus mykiss*). *Fresenius Environmental Bulletin* **14**, 18–22.
- Rotruer J.T. & Poue A.L. (1993) Se biochemical role as a component of glutathione peroxidase. *Science* **179**, 588–589.
- Roussyn I., Briviba K., Masumoto H. & Sies H. (1996) Selenium-containing compounds protect DNA from single-strand breaks caused by peroxynitrite. *Archives of Biochemistry and Biophysics* **330**, 216–218.
- Şahin M., Sağdıç G., Elmas O., Akpınar D., Derin N., Aslan M., Agar A., Alıcıgüzel Y. & Yargıçoğlu P. (2007) Effect of chronic restraint stress and alpha-lipoic acid on lipid peroxidation and antioxidant enzyme activities in rat peripheral organs. *Pharmacological Research* **54**, 247–252.

- Shacter E., Williams J.A., Lim M. & Levine R.L. (1994) Differential susceptibility of plasma proteins to oxidative modification: examination by western blot immunoassay. *Free Radical Biology and Medicine* **17**, 429–437.
- Sies H. (1999) Glutathione and its role in cellular functions. *Free Radical Biology and Medicine* **27**, 916–921.
- Soli N.E. (1980) Chronic copper poisoning in sheep. A review of the literature. *Nordisk veterinaer medicin* **32**, 75–89.
- Srivastava S., Mishra S., Tripathi R.D., Dwivedi S. & Gupta K.D. (2006) Copper-induced oxidative stress and responses of antioxidants and phytochelatins in *Hydrilla verticillata* (Lf) Royle. *Aquatic Toxicology* **80**, 405–415.
- Stadtman E. & Oliver C. (1991) Metal-catalyzed oxidation of proteins. Physiological consequences. *Journal of Biological Chemistry* **266**, 2005–2008.
- Tran D., Moody A.J., Fisher A.S., Foulkes M.E. & Jha A.N. (2007) Protective effects of selenium on mercury-induced DNA damage in mussel haemocytes. *Aquatic Toxicology* **84**, 11–18.
- Trevisan R., Mello D.F., Fisher A.S., Schuwerack P.M., Dafre A.L. & Moody A.J. (2011) Selenium in water enhances antioxidant defenses and protects against copper-induced DNA damage in the blue mussel *Mytilus edulis*. *Aquatic Toxicology* **101**, 64–71.
- Upadhyay R. & Panda S.K. (2010) Zinc reduces copper toxicity induced oxidative stress by promoting antioxidant defense in freshly grown aquatic duckweed *Spirodela polyrrhiza* L. *Journal of Hazardous Materials* **175**, 1081–1084.
- Wang W. & Ballatori N. (1998) Endogenous glutathione conjugates: occurrence and biological functions. *Pharmacological Reviews* **50**, 335–355.
- Winterbourn C.C. (1982) Superoxide-dependent production of hydroxyl radicals in the presence of iron salts (Letter). *Biochemical Journal* **205**, 463.
- Zhang W., Mai K., Xu W., Tan B., Ai Q., Liufu Z., Ma H. & Wang X. (2007) Interaction between vitamins A and D on growth and metabolic responses of abalone *Haliotis discus hannai* Ino. *Journal of Shellfish Research* **26**, 51–58.