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Protective effects of dietary selenium on abalone *Haliotis discus hannai* against the toxicity of waterborne cadmium

Yanlin Guo[1*] | Yanju Lei[2*] | Wei Xu[1] | Yanjiao Zhang[1] | Huihui Zhou[1] | Wenbing Zhang[1,3] | Kangsen Mai[1,3]

[1]The Key Laboratory of Mariculture (Ministry of Education), The Key Laboratory of Aquaculture Nutrition and Feeds (Ministry of Agriculture), Fisheries College, Ocean University of China, Qingdao, China

[2]Collaborative Innovation Center for Efficient and Health Production of Fisheries in Hunan Province, Key Laboratory of Health Aquaculture and Product Processing in Dongting Lake Area of Hunan Province, Zoology Key Laboratory of Hunan Higher Education, Hunan University of Arts and Science, Hunan Changde, China

[3]Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao, China

Correspondence
Wenbing Zhang, The Key Laboratory of Mariculture (Ministry of Education), The Key Laboratory of Aquaculture Nutrition and Feeds (Ministry of Agriculture), Fisheries College, Ocean University of China, Qingdao 266003, China.
Email: wzhang@ouc.edu.cn

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Abstract

This study was conducted to investigate protective effects of dietary selenium (Se) on abalone *Haliotis discus hannai* Ino against the toxicity of cadmium (Cd). A 60-day feeding trial was conducted with abalone (initial weight: 3.17 ± 0.01 g), which were exposed to 0.34 mg/L of waterborne Cd. During a feeding trial, abalone were fed graded levels of Se at 0.10 (controls), 1.31, and 4.20 mg/kg diet respectively. Results showed that there was no significant difference in specific growth rate and survival rate of abalone among the three treatments. Compared with the controls, dietary Se significantly decreased Cd concentrations in serum, muscle, mantle, gill, mantle, and hepatopancreas of abalone. Besides, compared with the controls, dietary Se significantly increased metallothionein concentration in the hepatopancreas of abalone. Additionally, compared with the controls, dietary Se significantly decreased concentrations of malondiadehyde and protein carbonyl in hepatopancreas of abalone. Meanwhile, compared with the controls, dietary Se significantly increased activities of glutathione peroxidase, thioredoxin reductase, and thioredoxin peroxidase, and concentration of glutathione in the hepatopancreas of abalone. Based on the data above, in abalone, dietary Se showed protective effects against waterborne Cd.

KEYWORDS
abalone, anti-oxidation, cadmium, *Haliotis discus hannai*, selenium, toxicity

1 | INTRODUCTION

Heavy metal pollution attracts more attention because of its toxicity, biological amplification, and bioaccumulation (Duruibe & Ogwuegbu, 2007). Cadmium (Cd) is a nonessential metal element, with high toxicity, which tends to accumulate in organisms and is difficult to be degraded (Maryanski, Kramarz, & Niklinska, 2002). It has been reported that Cd accumulation in an organism could induce reactive oxygen species (ROS) production (Galán et al., 2001; Tamás, Valentovicová, Halusková, Huttová, & Mistrík, 2009), and then induce oxidative damage to lipid and protein (Dandona et al., 1996; Kim, Je, & Kim, 2007). The oxidative damage induced by Cd is one of the main reasons for its toxic effects on organisms, such as sea bass *Dicentrarchus labrax* (Liu, Qu, & Kadiiska, 2009). Hence, it is necessary to reduce the oxidative damage induced by Cd to avoid its toxicity.

Selenium (Se) is an important mineral element in organisms, which has been considered to be a natural antioxidant to heavy metal toxicity (Frost & Lish, 1975). It plays important roles in the

*These authors contributed equally to this work.

synthesis of metallothionein (MT), which could combine with heavy metals and then reduce the toxicity (Dabrio et al., 2002; Tran, Moody, Fisher, Foulkes, & Jha, 2007). In addition, Se is an important component of glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) (Wu, Huang, & Xu, 2003). In organisms, antioxidative enzyme such as superoxide dismutase (SOD), GPx, and TrxR, and nonenzymatic antioxidants, such as glutathione (GSH), play important roles in protecting cells from oxidative damage induced by heavy metals (Finkel & Holbrook, 2000; Guo et al., 2017). A previous study has demonstrated that Se plays important roles in protecting rat's liver and kidney from oxidative damage caused by Cd exposure (Ognjanović et al., 2008). Up to now, there are no published data on the protective effects of Se on the toxicity of Cd in shellfish.

Abalone *Haliotis discus hannai* Ino is one of the most commercially important marine gastropod (Lei et al., 2016). In recent years, abalone culture has suffered from severe heavy metal pollution and oxidative damage (Lei et al., 2015; Silva-Aciares, Moraga, & Riquelme, 2013). Hence, it is necessary to improve the capacity of abalone against oxidative damage induced by heavy metal stress. The present study investigated effects of dietary Se on heavy metal accumulation and antioxidative capacity of abalone after waterborne Cd exposure. It provides basic data for better understanding of protective effects of Se on abalone under Cd stress.

2 | MATERIALS AND METHODS

2.1 | Experimental diets preparation

The basal diet formulation was based on that of Wu et al. (2010) 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 optimal growth for abalone *H. discus hannai* Ino (Mai, Mercer, & Donlon, 1995a, 1995b). Procedures for diet preparation and storage were prepared as previously described (Zhang et al., 2007). Proximate analyses of crude protein, crude lipid, and crude ash in diets were conducted following the standard procedures (AOAC, 1995). The $Na_2SeO_3{\cdot}5H_2O$ was used as the dietary Se source to design three supplemented levels of dietary Se. They were 0 (controls), 1.5, and 4.5 mg/kg respectively. The final concentrations of dietary Se were 0.10, 1.31, and 4.20 mg/kg, respectively, as determined by hydride generation atomic absorption spectrophotometer (HG-AAS) (Wang et al., 2012).

TABLE 1 Ingredients and compositions of the basal diet

Ingredients	Contents (%)
Casein[a]	25.00
Gelatin[b]	6.00
Dextrin[b]	33.50
Carboxymethyl cellulose[b]	5.00
Sodium alginate[b]	20.00
Vitamin mix[c]	2.00
Mineral mix[d]	4.50
Choline chloride[b]	0.50
SO/MFO[e]	3.50
Proximate analysis (dry weight %)	
Crude protein	29.41
Crude lipid	3.26
Crude ash	10.01

[a]Sigma Chemical, St Louis, MO, USA.
[b]Shanghai Chemical, Shanghai, China.
[c]Vitamin mix, each 1,000 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, 4,000 mg; biotin, 12 mg; ascorbic acid, 4,000 mg; B12, 0.18 mg; vitamin E, 450 mg; menadione, 80 mg; retinol acetate, 100,000 IU; cholecalciferol, 2,000 IU.
[d]Mineral mix, each 1,000 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; $ZnSO_4{\cdot}7H_2O$, 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.
[e]Soybean oil and menhaden fish oil (1:1).

2.2 | Feeding trial

Three hundred and sixty healthy juvenile abalone (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 laboratory conditions for 2 weeks. Based on the results of Lei et al. (2015), the waterborne Cd concentration was set as 0.34 mg/L from $CdCl_2{\cdot}2.5H_2O$. The feeding trial was conducted in tanks (100 L) in a static water system for 60 days. After measuring body weight, abalone were assigned to a static water system using a completely randomized design with three triplicated treatments. Half of the water in the tanks was exchanged with fresh seawater twice daily. Each replicate (tank) consisted of 40 abalones. Diets were hand-fed to abalone to satiation once daily at 18:00. Every morning, faeces and uneaten feed were removed to maintain the water quality. During the 60-day feeding trial, water temperature was maintained at 17–20°C, salinity 22–27 g/L, pH 7.4–7.9, and dissolved oxygen was not below 6 mg/L. The concentration of Se in seawater was determined to be 0.46 μg/L. Photoperiod regime during the feeding trial was 12 hr light:12 hr dark.

2.3 | Sample collection and analysis

Before sampling, all the abalone were fasted for 72 hr. Following this, all abalone were removed from the tanks, then weighed (each replicate was weighted as a group), and counted. 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. Haemolymph was obtained by use of syringes and needles from the adductor muscles. Serum was collected and immediately frozen in liquid nitrogen. Serum, shell, muscle, mantle, gill, and hepatopancreas samples per tank were pooled for analysis. The hepatopancreas was stored at

−80°C. The shell, muscle, mantle, gill, and serum were stored at −20°C.

The shell, muscle, mantle, gill, and hepatopancreas samples were lyophilized for 12 hr. Powdered samples (about 100 mg) or 500 μl of serum were acidified with 10 ml perchloric acid. Cadmium 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).

The concentration of MT in hepatopancreas was determined using enzyme-linked immunosorbent assay Kit (ELISA Kit; Nanjing Jiancheng Bioengineering Institute, Nanjing, China). A microplate was coated with purified MT antibody, which was made into a solid-phase antibody. Then, the MT was added into micropore coated with monoclonal antibody and combined with MT antibody which was marked by horse radish peroxidase (HRP), resulting in an antibody–antigen–solid-phase antibody complex. The complex was then reacted with 3, 3′, 5, 5′-tetramethyl benzidine (TMB) after washing thoroughly. The TMB turned blue due to the catalysis of HRP, and finally turned yellow under the effect of acid. The OD value was determined under the 450-nm wavelength. The concentration of MT was calculated according to the standard curve.

Concentrations of malondiadehyde (MDA), protein carbonyl (PC), and GSH, and activities of SOD, catalase (CAT), GPx, glutathione S-transferase (GST), TrxR, and thioredoxin peroxidase (TrxP) in hepatopancreas were measured according to the methods of Lei et al. (2016).

Total RNA samples were isolated from the hepatopancreas using RNAiso Plus Kit (9109; Takara Biotech, Dalian, China). The quality of RNA was assessed using agarose gel electrophoresis. Single-stranded cDNA was prepared from total RNA by reverse transcription using a PrimeScripte RT reagent Kit (RR047A; Takara Biotech). Primers used to amplify the target genes are listed in Table 2. Based on the results of the previous study (Wu, Zhang, Mai, Xu, & Zhong, 2011), real-time PCR assays were carried out in a quantitative thermal cycler (Mastercycler ep realplex; Eppendorf, Germany) in a final volume of 25 μl containing 12.5 μl 2× SYBR Green Realtime PCR Master Mix (Takara, Japan), 2 μl each of primers (10 μmol/L), and 1 μl of cDNA mix. β-actin was used as an endogenous reference to normalize the template amount. Real-time PCR temperature profile was 94°C for 2 min followed by 35 cycles of 5 s at 94°C, 15 s at 58°C, 20 s at 72°C. To confirm the specificity and purity of all PCR products, melt curve analysis was carried out after amplification: 94°C for 15 s and 59°C for 20 s. Fluorescent data were acquired during each annealing phase. During the detection, each sample was run in quartic, and PCR-grade water which replaced the template was the negative control. Results of gene expression were analysed using the $2^{-\Delta\Delta CT}$ method according to Livak and Schmittgen (2001).

TABLE 2 Real-time PCR primer sequences

Primer	Sequence (5′–3′)	Accession number
MT (forward)	ATGTCCAGTCCCCAAGGC	KT895222.1
MT (reverse)	CCACACTCGCAAGAACCTG	
MTF-1 (forward)	CGGCTGTGAGAAGTCTTTCAAC	KT895224.1
MTF-1 (reverse)	TGTCCGAATGTGTTTACGAAGATC	
β-actin (forward)	ACTCATTCACCACCACCG	AY380809.1
β-actin (reverse)	GGATGAAGAGGCAGCAGTAG	

Note. MT: metallothionein; MTF-1: Metal transcription factor-1.

2.4 | Calculations and statistical analysis

Growth was expressed as the specific growth rate (SGR, % per day). The calculation formula is as follows:

$$\mathrm{SGR}(\%/\mathrm{day}) = 100 * (\mathrm{Ln}(\mathrm{Final\ body\ weight}) - \mathrm{Ln}(\mathrm{Initial\ body\ weight}))/\mathrm{days}.$$

Statistical analysis was performed using SPSS 16.0 (SPSS, Chicago, IL, USA) and Microsoft Excel 2010. Data were analysed by one-way ANOVA. When significant differences ($p < 0.05$) were found, means were compared using Tukey's test. All the data were presented as means ± *SE*.

3 | RESULTS

3.1 | Growth performance

As shown in Table 3, there was no significant difference in SGR among three treatments, and the highest SGR was found in 1.31 mg Se/kg treatment. The survival rate had no significant difference among the three treatments, and the 4.20 mg Se/kg treatment showed the highest survival rate (93.33%).

3.2 | Cd concentration in organs

As shown in Table 4, dietary Se significantly influenced Cd concentration in serum ($p < 0.05$), muscle ($p < 0.05$), mantle ($p < 0.05$), gill ($p < 0.05$), and hepatopancreas ($p < 0.05$). In serum, mantle, gill, and hepatopancreas, the Cd concentration in 1.31 and 4.20 mg Se/kg treatment was significantly lower than those in the control treatment ($p < 0.05$). In muscle, the 4.20 mg Se/kg treatment showed the significantly lower Cd concentration than the control treatment

TABLE 3 Effects of dietary selenium on growth and survival of abalone *Haliotis discus hannai* exposed to waterborne Cd for 60 days (mean ± *SE*, $n = 3$)

Dietary selenium (mg/kg)	Initial weight (g)	Final weight (g)	Specific growth rate (%)	Survival rate (%)
0.10	3.17 ± 0.01	3.88 ± 0.09	0.34 ± 0.04	92.50 ± 1.44
1.31	3.17 ± 0.01	4.01 ± 0.14	0.39 ± 0.05	90.83 ± 3.01
4.20	3.15 ± 0.01	3.83 ± 0.10	0.33 ± 0.04	93.33 ± 2.21
One-way ANOVA				
p Value	0.425	0.690	0.563	0.748
F value	0.989	0.537	0.632	0.304

TABLE 4 Effects of dietary selenium on Cd accumulation in tissues of abalone *Haliotis discus hannai* exposed to waterborne Cd for 60 days (mean ± *SE*, *n* = 3)

Dietary Se (mg/kg)	Serum (μg/ml)	Shell (μg/g)	Muscle (μg/g)	Mantle (μg/g)	Gill (μg/g)	Hepatopancreas (μg/g)
0.10	1.94 ± 0.08^{a}	19.49 ± 3.69	17.34 ± 1.57^{a}	37.32 ± 0.46^{a}	69.66 ± 3.57^{a}	418.84 ± 21.31^{a}
1.31	1.33 ± 0.10^{b}	16.39 ± 1.92	14.85 ± 1.85^{ab}	33.19 ± 0.02^{b}	56.42 ± 2.15^{b}	306.85 ± 9.44^{b}
4.20	1.38 ± 0.12^{b}	21.14 ± 3.35	11.35 ± 1.35^{b}	32.66 ± 0.65^{b}	56.55 ± 2.57^{b}	253.07 ± 19.40^{b}
One-way ANOVA						
p Value	0.009	0.573	0.012	0.000	0.025	0.000
F value	11.528	0.611	10.063	30.922	7.246	41.251

Note. 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.

($p < 0.05$). However, the 1.31 mg Se/kg treatment had no significant difference with the control treatment.

3.3 | Concentration and mRNA level of metallothionein in hepatopancreas

As shown in Figure 1, dietary Se significantly influenced MT concentration in hepatopancreas. The MT concentrations in 1.31 and 4.20 mg Se/kg treatment were significantly higher than that in the control treatment ($p < 0.05$). There is no significant difference between 1.31 and 4.20 mg Se/kg treatment. However, dietary Se had no significant influence on mRNA levels of MT and metal-responsive transcription factor 1 (MTF-1) in hepatopancreas.

3.4 | Antioxidation-related parameters in hepatopancreas

As shown in Table 5, dietary Se significantly influenced MDA ($p < 0.05$) and PC ($p < 0.05$) concentrations. The MDA and PC concentrations in 1.31 and 4.20 mg Se/kg treatment were significantly lower than those in the control treatment ($p < 0.05$). There was no significant difference in concentrations of MDA and PC between 1.31 and 4.20 mg Se/kg treatment.

As shown in Table 6, dietary Se had significant influence on activities of GPx ($p < 0.05$), TrxR ($p < 0.05$) and TrxP ($p < 0.05$), and concentration of GSH ($p < 0.05$) in the hepatopancreas. The activities of GPx, TrxR, and TrxP, and concentration of GSH in 1.31 and 4.20 mg Se/kg treatment were significantly higher than those in the control treatment ($p < 0.05$). There was no significant difference in activities of GPx, TrxR, and TrxP, and concentration of GSH between the 1.31 and 4.20 mg Se/kg treatments. Besides, there was no significant difference in activities of SOD, CAT, and GST among the three treatments.

4 | DISCUSSION

In the present study, compared with the control treatment, 1.31 and 4.20 mg/kg of dietary Se caused a significant reduction in Cd concentration in serum, mantle, gill, and hepatopancreas of abalone. Treatment with 4.20 mg/kg of dietary Se caused a significant reduction in Cd concentration in muscle. Similarly, it has been reported that dietary Se decreased Cd concentration in the body of Nile tilapia *Oreochromis niloticus* (L.) (Abdel-Tawwab & Wafeek, 2010). MT is a low molecular weight protein with high affinity for metal ions, which is widely existed in organisms (Nordberg & Kojima, 1979). It has been reported that MT may reduce toxicity of heavy metals, such as mercury (Hg), copper (Cu), and Cd, through combining with them (Dabrio et al., 2002). Moreover, it has been reported that Se may induce the synthesis of MT in mussel haemocytes (Tran et al., 2007). In present study, 1.31 and 4.20 mg/kg of dietary Se significantly increased MT concentration in hepatopancreas. It is suggested that Se plays important roles in defending against the toxicity of Cd, which might be related with MT in abalone.

Studies revealed that Cd stress could lead to overproduction of ROS in organism, which could damage lipid and protein (Stohs, Bagchi, Hassoun, & Bagchi, 2000). MDA is a general indicator for lipid peroxidation (Gaweł, Wardas, Niedworok, & Wardas, 2004). According to a previous study, after a 33-day Cd stress, the lipid peroxidation level in liver of Atlantic croaker *Micropogonias undulates* was increased (Thomas & Wofford, 1993). In the present study, 1.31 and 4.20 mg/kg of dietary Se significantly decreased MDA concentrations in the hepatopancreas of abalone. Similarly, a previous study on rats showed that Se significantly decreased MDA concentration in kidney under Cd stress (El-Sharaky, Newairy, Badreldeen, Eweda, & Sheweita, 2007). These data suggested that Se plays an important role in decreasing the lipid peroxidation level induced by Cd. Besides, excessive ROS could lead to modifications of nonenzymatic protein, such as PC, which is considered to be a biomarker of oxidative damage (Shacter, Williams, Lim, & Levine, 1994). According to a previous study, the PC concentration in haemocytes of mussels was significantly increased after Cd exposure (Kaloyianni et al., 2009). In the present study, compared with the control treatment, 1.31 and 4.20 mg Se/kg treatment significantly decreased PC concentration in the hepatopancreas of abalone. The result is coincident with a previous study, in which Se significantly decreased PC concentration in rat erythrocytes after Cd exposure (Soudani et al., 2011). All the data above suggested that Se play sn important role in protecting hepatopancreas from oxidative damage induced by Cd.

In animals, ROS could be removed by antioxidative enzymes (e.g., GPx) and nonenzymatic antioxidants (e.g., GSH) (Martínez-

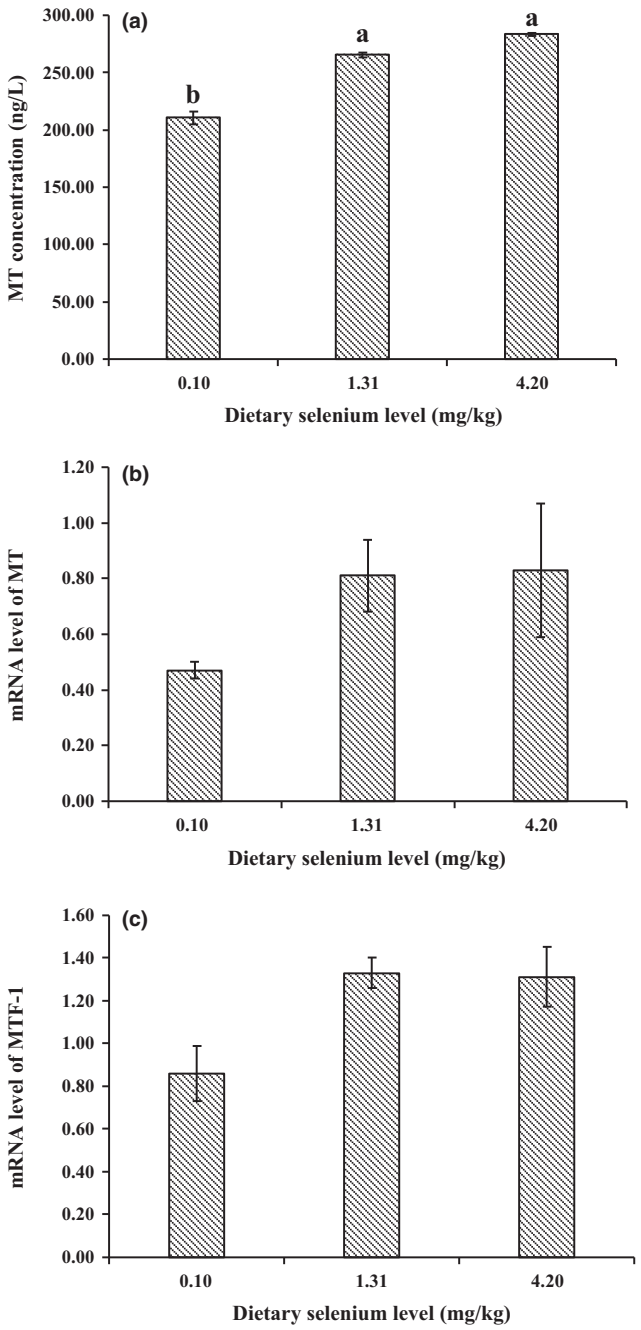

FIGURE 1 Metallothionein (MT) concentration (a) and Relative MT (b) and MTF-1 mRNA (c) levels in hepatopancreas of abalone *Haliotis discus hannai* Ino fed dietary Se under waterborne Cd for 60 days. All values were presented as the mean ± *SE* (n = 3). Means sharing a common superscript letter were not significantly different ($p > 0.05$) as determined by Tukey's test

Álvarez, Morales, & Sanz, 2005; Nugroho & Fotedar, 2013). Selenium is considered to be a natural antioxidant because it is an important component of GPx and TrxR in organisms (Maleki, Safavi, & Doroodmand, 2005; Rotruck et al., 1973). In the present study, compared with the control treatment, 1.31 and 4.20 mg/kg of dietary Se significantly increased the activities of GPx, TrxR, and TrxP in hepatopancreas. A previous study showed a similar result that Se significantly

TABLE 5 Effects of dietary selenium on the oxidative parameters in hepatopancreas of abalone *Haliotis discus hannai* Ino exposure to waterborne Cd for 60 days (mean ± *SE*, n = 3)

Treatments (mg/kg)	MDA	PC
0.10	24.11 ± 0.29[a]	8.67 ± 0.23[a]
1.31	22.07 ± 0.10[b]	4.35 ± 0.32[b]
4.20	21.63 ± 0.53[b]	5.79 ± 0.43[b]
One-way ANOVA		
p Value	0.005	0.000
F value	14.046	42.939

Notes. Means in the same column sharing a common superscript letter were not significantly different ($p > 0.05$) as determined by Tukey's test. MDA: malondiadehyde (nmol/mg Prot); PC: protein carbonyl (nmol/mg Prot).

increased GPx activity in serum of rat (Elboshy, Risha, Abdelhamid, Mubarak, & Hadda, 2015). Besides, El-Sharaky et al. (2007) demonstrated that Se significantly increased activity of TrxR in kidney of rats. These results were coincident with the present study. In addition to antioxidative enzyme, nonenzymatic antioxidants, such as GSH, also play important roles in decreasing oxidative damage (Nugroho & Fotedar, 2013). Glutathione are considered to be the most important antioxidant in cytophylaxis (Meister & Anderson, 1983; Segner & Braunbeck, 1998). It has been reported that GSH is a tripeptide molecule abundant with sulphydryl and it could chelate with metal ion to alleviate toxicity of heavy metals (Burton et al., 1995; Foulkes, 1993; Ochi, Otsuka, Takahashi, & Ohsawa, 1988). In the present study, 1.31 and 4.20 mg/kg of dietary Se significantly increased GSH concentration in hepatopancreas. Similarly, Se significantly increased GSH concentration in kidney of rats under Cd stress (El-Sharaky et al., 2007). These data suggested that dietary Se could reduce oxidative damage induced by Cd through increasing the activities of antioxidative enzymes and the concentration of nonenzymatic antioxidants in hepatopancreas of abalone.

In present study, compared with 1.31 mg/kg of dietary Se, 4.20 mg/kg of dietary Se had no negative effects on ability against the waterborne Cd. The possible reason was that there might be a higher Se requirement of abalone defending against waterborne Cd. It has been reported that dietary arginine requirement of young grass carp based on ability against waterborne copper (17.26 g/kg) (Wang, Feng, et al., 2015) is higher than that based on growth performance (13.45 g/kg) (Wang, Liu, et al., 2015). Besides, the dietary iron requirement of young grass carp based on ability against *Aeromonas hydrophila* (87.03 mg/kg) is also higher than that based on growth performance (75.65 mg/kg) (Guo et al., 2017). Accordingly, it is hypothesized that Se requirement of abalone under stress might be higher than that based on growth performance. According to the previous study, dietary Se requirement based on weight gain was 1.408 mg/kg for abalone (Wang et al., 2012). On the basis of these data, it is suggested that in addition to the Se that meet the normal growth, abalone need more Se to defend against waterborne Cd. This could explain that 4.20 mg/kg of dietary Se had no negative

TABLE 6 Effects of dietary selenium on antioxidative parameters in hepatopancreas of abalone *Haliotis discus hannai* Ino exposure to waterborne Cd for 60 days (mean ± *SE*, *n* = 3)

Dietary Se (mg/kg)	SOD	CAT	GPx	GST	GSH	TrxR	TrxP
0.10	10.64 ± 0.75	3.25 ± 0.35	4.76 ± 0.27[b]	2.53 ± 0.03	5.88 ± 0.14[b]	2.51 ± 0.03[b]	5.38 ± 0.16[b]
1.31	10.30 ± 0.50	4.18 ± 0.70	9.03 ± 1.12[a]	3.05 ± 0.23	7.64 ± 0.06[a]	2.97 ± 0.02[a]	6.61 ± 0.28[a]
4.20	10.47 ± 0.42	4.54 ± 0.08	8.00 ± 0.38[a]	2.90 ± 0.05	7.21 ± 0.11[a]	2.84 ± 0.07[a]	7.17 ± 0.30[a]
One-way ANOVA							
p Value	0.917	0.198	0.012	0.089	0.000	0.001	0.007
F value	0.088	2.144	10.174	3.730	71.627	28.729	12.840

Notes. Means in the same column sharing a common superscript letter were not significantly different ($p > 0.05$) as determined by Tukey's test.
CAT: catalase (U/mg Prot); GPx: glutathione peroxidase (U/mg Prot); GSH: glutathione (mg/g Prot); GST: glutathione S-transferases (U/mg Prot); SOD: total superoxide dismutase (U/mg Prot); TrxP: thioredoxin peroxidase (U/mg Prot); TrxR: thioredoxin reductase (mU/L).

effects on abalone against waterborne Cd. However, it requires further investigation.

5 | CONCLUSION

Generally, the present study confirmed that dietary Se plays important roles in abalone against Cd toxicity. Firstly, a supplement of dietary Se decreased Cd accumulation in serum, mantle, gill, and hepatopancreas of abalone, which might be related to the increased concentration of MT. Secondly, a supplement of dietary Se decreased MDA and PC concentrations in hepatopancreas of abalone, which might be related to increased activities of GPx, TrxR, and TrxP, and concentration of GSH. Finally, based on the present results, 1.31 mg/kg of dietary Se is suggested to help abalone defend against waterborne Cd toxicity.

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ORCID

Yanjiao Zhang http://orcid.org/0000-0002-7471-9021
Wenbing Zhang http://orcid.org/0000-0002-6546-3087

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