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Protective effects of dietary α-lipoic acid on abalone *Haliotis discus hannai* against the oxidative damage under waterborne cadmium stress

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Abstract

A 60-day feeding trial was conducted to investigate the protective effects of dietary α-lipoic acid (LA) on juvenile abalone *Haliotis discus hannai* against the oxidative damage under waterborne cadmium (Cd) stress. Three experimental diets were formulated with 0 (Control), 700 (LA-700) and 2100 (LA-2100) mg/kg supplement of LA respectively. Each diet was randomly assigned to three replicates of 50 abalones (initial body weight: 3.17 ± 0.01 g), which were exposed to 0.35 mg/L of waterborne Cd. Results showed that abalones' Cd bioaccumulation in the serum, mantle, gill, muscle and hepatopancreas were significantly reduced in LA supplement groups. Besides, in hepatopancreas, the activities of antioxidative enzymes and concentration of glutathione (GSH) in LA supplement group were significantly higher than those in control. Meanwhile, in hepatopancreas, LA-2100 group showed a significantly decreased malondialdehyde (MDA) concentration and the protein carbonyls in LA supplement groups were significantly lower than those in control. Diets with LA supplementation significantly upregulated the mRNA expression of HdhMTF-1 (*H. discus hannai* metal-responsive transcription factor-1) and HdhMT (*H. discus hannai* metallothionein), and elevated metallothionein (MT) concentrations in hepatopancreas. In conclusion, it was suggested that dietary LA could alleviate Cd-induced oxidative damage through enhancing antioxidative enzyme activities and upregulating the HdhMTF-1-mediated HdhMT transcription and synthesis.

KEYWORDS
antioxidation, cadmium, diet, *Haliotis discus hannai*, toxicity, α-lipoic acid

1 | INTRODUCTION

Cadmium (Cd) is one of the most common toxic heavy metals with the greatest potential hazard to the environment, and it poses a serious threat to animals' health as it accumulates in the environment through contaminated growing waters (Huang, Ke, & Wang, 2008; Xu, Feng, Jeffrey, Shi, & Morel, 2008). Due to the bioaccumulation and toxicity of Cd in liver and kidney, elevated Cd concentrations in aquatic animals inhibit the antioxidative system and induce the over-production of reactive oxygen species (ROS), which results in the disruption of redox homeostasis (Huang, Guo, Ke, & Wang, 2010; Shi, Sui, Wang, Luo, & Ji, 2005). Furthermore, the intracellularly excess ROS induced by Cd can contribute to the damage of tissue macromolecules, such as proteins and lipids (Amamou et al., 2015).

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Metallothionein (MT) belongs to a superfamily of metalloproteins possessing a unique type of sulphur-based metal clusters. It presents in a range of aquatic organisms. In the detoxification of heavy metals, MT is important in the chelating heavy metal ions, especially Cd and Zn (Sato & Bremner, 1993; Vašák, 2005). As it was shown, MT concentration can be significantly increased in Nile tilapia (*Oreochromis niloticus* L.), common carp (*Cyprinus carpio* L.) and abalone (*Haliotis diversicolor*) when exposed to Cd (Abdel-Tawwab & Wafeek, 2014; Huang et al., 2010; Huang, Zhang, Chen, Zhuang, & Wang, 2007). In response to heavy metal ions or oxidative stress, metal-responsive transcription factor-1 (MTF-1), which is the most comprehensively studied as an upstream regulatory element of MT, is also involved in the mediation of intracellular metal ion balance (Dong, Chen, Qi, Dou, & Wang, 2015; Günther, Davis, Georgiev, & Schaffner, 2012). Additionally, MTF-1 was also found to be Cd inducible in *Haliotis discus hannai* (Lee & Nam, 2017).

Alpha-lipoic acid (LA), a naturally occurring sulfhydryl compound, is a well-known cofactor of mitochondrial dehydrogenases and decarboxylases with excellent antioxidant properties (Odabasoglu et al., 2011). Both LA and its reduced form, dihydrolipoic acid (DHLA) act as antioxidants in hydrophilic and lipophilic environments (Park, Terjesen, Tesser, Portella, & Dabrowski, 2006). In addition, LA can directly scavenge ROS induced by oxidative stress in vivo, and DHLA is able to regenerate endogenous antioxidants and to repair oxidative damage (Biewenga, Haenen, & Bast, 1997). In fish, LA was shown to reduce muscle lipid peroxidation (Kütter, Monserrat, Primel, Caldas, & Tesser, 2012), and to improve the detoxification and antioxidative capacity (Monserrat et al., 2008).

Abalone *H. discus hannai* is the most commercial and important marine gastropod cultured in China. Previous study confirmed that dietary LA can promote the growth and stimulate the antioxidative capacity of abalone *H. discus hannai* (Zhang et al., 2010). The aim of the present study was to elucidate the effects of LA on detoxification of Cd in abalone. It provides basic data for better understanding of protective effects of LA on abalone under Cd stress.

2 | MATERIALS AND METHODS

2.1 | Experimental diets

The basal diet formulation was based on Wu et al. (2010) with some modifications (Table 1). The diet was formulated with purified ingredients to provide 294.10 g/kg crude protein from casein and gelatin, and 32.60 g/kg crude lipid from soybean oil and menhaden fish oil (1:1). Based on the basal diet, two LA-supplemented diets were formulated by including 700 and 2,100 mg/kg of LA respectively. Procedures for diet preparation and storage were 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).

TABLE 1 Ingredient and proximate composition of the basal diet

Ingredient	Contents (g/kg)
Casein[a]	250.00
Gelatin[b]	60.00
Dextrin[b]	335.00
CM-cellulose[b]	50.00
Sodium alginate[b]	200.00
Vitamin mix[c]	20.00
Mineral mix[d]	45.00
Choline chloride[b]	5.00
SO/MFO[e]	35.00
Proximate analysis (dry weight g/kg)	
Moisture	298.23
Crude protein	294.14
Crude lipid	32.63
Crude ash	100.14

[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; $MgSO_4.7H_2O$, 6.0 g; $NaH_2PO_4.2H_2O$, 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; $ZnSO_4.7H_2O$, 141.2 mg; $MnSO_4{\cdot}H_2O$, 64.8 mg; $CuSO_4.5H_2O$, 12.4 mg; $CoCl_2.6H_2O$, 0.4 mg; KIO_3, 1.2 mg. [e]Soybean oil and menhaden fish oil (1:1).

2.2 | Feeding trial

Abalone juveniles were collected from a spawning at Laoshan Fisheries, Qingdao, China. Prior to the start of feeding trial, abalones were acclimated to the laboratory conditions for 2 weeks. Four hundred and fifty healthy abalones (initial body weight: 3.17 ± 0.01 g) were then randomly assigned to nine tanks (100 L) with 50 individuals per tank. Each diet was assigned to three tanks once daily at 18:00. During the feeding trial, uneaten feeds were removed at 8:00 the next morning from the tank to maintain the quality of seawater.

Based on the results of previous study (Lei et al., 2015), the semi-lethal concentration of waterborne Cd to abalone is 2.848 mg/L. The present study chooses 1/8 of semi-lethal concentration as the experimental one. Before the feeding trial, a stock solution (1,000 mg/L) was prepared by dissolving $CdCl_2{\cdot}2.5H_2O$ in distilled water, then it was diluted with natural seawater to achieve a final Cd concentration of 0.35 mg/L and ultimately pumped into each tank. Seawater in each tank was changed at 8:00 every morning.

During the 2-month feeding trial, water temperature ranged from 18 to 21°C, pH 7.4–7.9, salinity 22–27 and dissolved oxygen higher than 7.0 mg/L. The photoperiod regime was 12 hr light: 12 hr dark.

2.3 | Sample collection

At the end of the feeding trial, animals were not fed for 3 days. The total number and body weight of abalone in each tank were counted and measured. The haemolymph was drawn by a syringe from the adductor muscles and stored in 1.5 ml centrifuge tube at 4°C for 4 hr. Serum was collected after centrifugation (4,000 *g*, 10 min) and stored at −80°C as separate aliquots until analysis. For gene expression analysis, six samples of hepatopancreas per tank were randomly collected. In addition, another four samples of serum, shell, muscle, mantle, gill and hepatopancreas per tank were also randomly collected for enzyme activity analysis. All samples were immediately frozen in liquid nitrogen and stored at −80°C. For analysis, serum, shell, muscle, mantle, gill and hepatopancreas samples per tank were pooled. All animal experiments complied with the National Institutes of Health guide for the care and use of Laboratory animals (http://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-use-of-laboratory-animals.pdf).

2.4 | Chemical analysis of diets

Proximate analyses of crude protein, crude lipid and crude ash in diets were conducted following the standard procedures (AOAC, 1995). Crude lipid was determined by ether extraction using Soxhlet (Extraction SystemB-811, BUCHI, Switzerland). Crude protein was determined by Kjeldahl method with a Kjeltec System (2300, FOSS, Sweden). Crude ash was determined by combustion at 550°C for 8 hr.

2.5 | Oxidation and antioxidation responses

The activities of superoxide dismutase (SOD), catalase (CAT), Se-dependent glutathione peroxidase (Se-GPx), glutathione S-transferases (GST), thioredoxin (Trx), thioredoxin reductase (TrxR) and thioredoxin peroxidase (TrxP) and the concentration of glutathione (GSH), malondialdehyde (MDA) and protein carbonyl were determined using the reported method (Lei et al., 2016).

The concentration of MT in hepatopancreas was determined using enzyme linked immunosorbent assay kit (ELISA) (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). 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. Then the complex was reacted with 3, 3′, 5, 5′- tetramethyl benzidine (TMB) after it was washed thoroughly. The TMB turned into blue under the catalyze of HRP, and finally turned into 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.

2.6 | Cadmium determination

The Cd 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).

2.7 | Real-time quantitative PCR

Hepatopancreas was ground into powder in liquid nitrogen and added to RNAiso Plus (9109; Takara Biotech, Dalian, China) to extract total RNA. The integrity of RNA was detected by electrophoresis using 10 g/kg denatured agarose gel and then assessed RNA concentration by Nano Drop® 2000 spectrophotometer (Thermo Fisher Scientific, USA). The first-strand cDNA was synthesized by reversely transcribing 1 μg total RNA using PrimeScript RT reagent Kit (RR047A; Takara Biotech, Dalian). Specific primers for target genes and housekeeping genes were synthesized by Sangon (Shanghai, China), and then assessed to determine the application efficiency. Primers sequences: HdhMT (GenBank accession no. KT895222.1) for Sense 5′-ATGTCCAGTCCCCAAGGC-3′, Anti-sense 5′-CCACACTCGCAAGAACCTG-3′, HdhMTF-1 (GenBank accession no. KT895224.1) for Sense 5′-CGGCTGTGAGAAGTCTTTCAAC-3′, Anti-sense 5′-TGTCCGAATGTGTTTACGAAGATC-3′ and β-actin (GenBank accession no. AY380809.1) for Sense 5′-ACTCATTC ACCACCACCG-3′, Anti-sense 5′-GGATGAAGAGGCAGCAGTAG-3′. Real-time PCR was conducted in a quantitative thermal cycler (Mastercycler ep realplex, Eppendorf, Germany). The amplification was performed in a total volume of 20 μl, containing 10 μl of EvaGreen Express 2 × qPCR MasterMix (MasterMix-ES, Applied Biological Materials, Canada), 1 μl of the cDNA product, 0.6 μl of each primer (10 mM) and 9.5 μl of diethylpyrocarbonate-treated water. The qRT-PCR conditions were: 95°C for 10 min and then 40 cycles of 95°C for 15 s, 58°C for 15 s and 72°C for 40 s. When PCR amplification was finished, melting curve analysis was performed to verify that only one PCR product was present in each of these reactions.

2.8 | Calculations and statistical analysis

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

$$\text{Specific growth rate (SGR, kg}^{-1}\text{ day}^{-1}) = 100 \times (\ln Wt - \ln Wi)/t$$

where Wt and Wi are final and initial abalone body weight (g) respectively; *t* is duration of the feeding trial (days).

Statistical analysis was performed using SPSS 16.0 (SPSS, Chicago, IL, USA) and Microsoft Excel 2010. Data were analysed by one-way ANOVA. Differences were regarded as significance when $p < 0.05$, and means were compared using Tukey's test. All data were expressed as means ± *SEM*.

3 | RESULTS

3.1 | Growth performance

As shown in Table 2, there were no significant differences in survival rate and SGR among all treatments ($p > 0.05$).

TABLE 2 Effects of dietary lipoic acid on growth and survival of juvenile abalone *Haliotis discus hannai* exposed to waterborne Cd for 60 days

Dietary LA (mg/kg)	Initial weight (g)	Final weight (g)	SGR (kg^{-1} day^{-1})	Survival (%)
0	3.14 ± 0.01	3.81 ± 0.12	3.21 ± 0.70	93.15 ± 2.35
700	3.16 ± 0.01	3.75 ± 0.04	2.82 ± 0.33	92.34 ± 4.37
2,100	3.17 ± 0.01	3.84 ± 0.07	3.23 ± 0.41	94.20 ± 2.12
One-way ANOVA				
p-value	0.079	0.716	0.674	0.815
F-value	3.998	0.353	0.421	0.212

Note. Values are presented as mean ± *SEM*, *n* = 3.

TABLE 3 Effects of dietary lipoic acid on Cd accumulation in tissues of juvenile abalone *Haliotis discus hannai* exposed to waterborne Cd for 60 days

Dietary LA (mg/kg)	Serum (μg/ml)	Shell (μg/g)	Muscle (μg/g)	Mantle (μg/g)	Gill (μg/g)	Hepatopancreas (μg/g)
0	2.23 ± 0.04^{a}	18.55 ± 2.08	15.37 ± 0.23^{a}	33.48 ± 1.20^{a}	77.42 ± 0.38^{a}	426.11 ± 3.63^{a}
700	1.45 ± 0.10^{b}	20.25 ± 4.58	12.71 ± 1.01^{ab}	21.76 ± 2.54^{b}	64.98 ± 2.83^{b}	209.93 ± 21.08^{c}
2,100	1.38 ± 0.15^{b}	25.89 ± 8.13	11.72 ± 0.27^{b}	24.81 ± 0.32^{b}	67.18 ± 2.07^{b}	321.08 ± 11.00^{b}
One-way ANOVA						
p-value	0.002	0.638	0.014	0.006	0.011	0.000
F-value	19.943	0.484	9.424	13.887	10.661	60.606

Note. Values are presented as mean ± *SEM*, *n* = 3. Values followed by different superscript letters in the same row are significantly different ($p < 0.05$).

3.2 | Cd concentration in tissues

Compared with the control group, both LA-700 and LA-2100 groups showed significantly decreased Cd concentration in serum, mantle, gill and hepatopancreas ($p < 0.05$), while Cd accumulation in muscle was significantly decreased only in LA-2100 group ($p < 0.05$). In addition, there was also a significant decrease in Cd concentration of hepatopancreas in LA-2100 group as compared with LA-700 Group ($p < 0.05$). However, the Cd concentration in shell was not significantly influenced by LA supplementation ($p > 0.05$; Table 3).

3.3 | Oxidation and antioxidation parameters

The activities of CAT, Se-GPx and GST in hepatopancreas in LA-700 group were significantly higher than that of control ($p < 0.05$), whereas the concentration of GSH showed a significantly elevated trend by dietary inclusion of 700 and 2,100 mg/kg LA. There were no significant differences between LA-700 group and LA-2100 group on the activities of CAT, Se-GPx and GST in hepatopancreas ($p > 0.05$). Compared with the control group, no differences were found in the effects of LA on the activities of SOD, Trx, TrxR and TrxP ($p > 0.05$; Table 4).

Dietary LA (700, 2,100 mg/kg) significantly decreased the protein carbonyl concentration in hepatopancreas. Meanwhile, only the treatment with 2,100 mg/kg of dietary LA showed significantly decreased MDA concentration compared with that in control ($p < 0.05$; Table 5).

In hepatopancreas, compared with the control group, diets with LA supplementation (700, 2,100 mg/kg) significantly upregulated the expression of HdhMTF-1 ($p < 0.05$) (Figure 1a). Abalone in LA-700 group showed a significantly increased HdhMT mRNA expression ($p < 0.05$). The expression of HdhMT in LA-2100 group showed no significant difference compared with the control group ($p > 0.05$) (Figure 1b). Moreover, there were significantly increased MT concentrations in dietary LA supplemented groups compared with the control group ($p < 0.05$) (Figure 1c).

4 | DISCUSSION

Alpha-lipoic acid and its reduced form DHLA can indirectly act to maintain cellular antioxidative status by either inducing the uptake or enhancing the antioxidative enzymes (Shay, Moreau, Smith, Smith, & Hagen, 2009). In the present work, dietary LA significantly increased the activities of CAT, Se-GPx and GST in hepatopancreas compared with the control. This is in accordance with the previous study in rat, in which it was found that dietary supplementation of LA (15 and 30 mg/kg) to cadmium-intoxicated rats (3 mg/kg) bolstered the antioxidants and antioxidative enzyme defenses (Sumathi, Baskaran, & Varalakshmi, 1996). Previous studies have already shown that LA can increase the activities of many antioxidative enzymes in aquatic organisms, such as GST, SOD and GPx in common carp *Cyprinus carpio*, Pacific white shrimp *Litopenaeus vannamei*, pompano *Trachinotus marginatus*, zebrafish *Danio rerio*

TABLE 4 Effects of dietary lipoic acid on antioxidant-related parameters in hepatopancreas of juvenile abalone *Haliotis discus hannai* exposed to waterborne Cd for 60 days

Dietary LA (mg/kg)	SOD	CAT	Se-GPx	GST	GSH	Trx	TrxR	TrxP
0	11.94 ± 0.16	3.01 ± 0.30^{b}	8.47 ± 0.30^{b}	1.99 ± 0.20^{b}	8.80 ± 0.12^{a}	298.47 ± 1.39	2.71 ± 0.11	6.52 ± 0.64
700	12.54 ± 0.38	3.96 ± 0.21^{a}	9.85 ± 0.18^{a}	3.57 ± 0.11^{a}	9.69 ± 0.22^{b}	294.76 ± 0.75	2.64 ± 0.24	6.81 ± 0.35
2,100	12.15 ± 0.81	3.52 ± 0.24^{ab}	10.03 ± 0.03^{a}	3.81 ± 0.03^{a}	10.44 ± 0.09^{c}	294.38 ± 1.90	2.85 ± 0.16	5.66 ± 0.43
One-way ANOVA								
p-value	0.724	0.01	0.003	0.000	0.001	0.161	0.7	0.298
F-value	0.341	10.707	18.237	55.115	24.042	2.515	0.379	1.494

Note. Values are presented as mean ± *SEM*, *n* = 3. Values followed by different superscript letters in the same row are significantly different ($p < 0.05$). SOD, total superoxide dismutase (U/mg Prot); CAT, catalase (U/mg Prot); GPX, glutathione peroxidase (U/mg Prot); GST, glutathione S-transferases (U/mg Prot); GSH, glutathione (mg/g Prot); Trx, thioredoxin (mU/L Prot); TrxR, thioredoxin reductase (mU/L Prot); TrxP, thioredoxin peroxidase (U/mg Prot).

TABLE 5 Effects of dietary lipoic acid (LA) on oxidant-related parameters in hepatopancreas of juvenile abalone *Haliotis discus hannai* exposed to waterborne Cd for 60 days

Dietary LA (mg/kg)	MDA (nmol/mg Prot)	Protein carbonyl (nmol/mg Prot)
0	23.70 ± 0.29^{a}	9.40 ± 1.11^{a}
700	21.12 ± 0.61^{a}	4.77 ± 0.06^{b}
2,100	16.04 ± 0.86^{b}	3.94 ± 0.05^{b}
One-way ANOVA		
p-value	0.000	0.002
F-value	37.83	20.767

Note. Values are presented as mean ± *SEM*, *n* = 3. Values followed by different superscript letters in the same row are significantly different ($p < 0.05$).

and abalone *H. discus hannai* (Amado et al., 2011). In the antioxidative network of LA, DHLA can mediate reduction of glutathione disulfide (GSSG) to GSH (Bast & Haenen, 2003). The present study showed the increased GSH concentration in abalone in LA-treated groups. Consequently, absorbed LA can be transformed to DHLA in abalone, thereby significantly increase intracellular GSH levels. Besides, all GPx isoforms are mainly associated with GST and use GSH as substrate for reduction of oxidized compounds (Almar, Otero, Santos, & Gallego, 1998). Accordingly, the activities of GST and Se-GPx could be improved with the increased GSH concentration as shown in Table 3. Generally, the present study suggested that LA can rescue the antioxidative capacity which was damaged by Cd in abalone hepatopancreas. These results were similar with our previous study, in which it was confirmed that the antioxidative capacity of juvenile abalone farmed in natural seawater (Cd-free) was improved by dietary LA supplementation (200–800 mg/kg; Zhang et al., 2010).

In present study, abalone fed with diets containing LA (700, 2,100 mg/kg) significantly upregulated the mRNA expression of HdhMT and the concentration of MT in abalone hepatopancreas. Besides, the mRNA expression of HdhMT was also significantly increased with elevated expression of HdhMTF-1, which was found to be Cd inducible in *H. discus hannai* (Lee & Nam, 2017). Similarly, dietary LA significantly increased the mRNA expression of metallothioneins (*Mt1a* and *Mt2A*) in rat liver (Ide, 2014). It has also been found that mouse metallothioneins (*Mt1* and *Mt2*) are overwhelming in response to transcriptional regulation of MTF-1 when exposed to Cd (Wimmer, Wang, Georgiev, & Schaffner, 2005). MT induced by heavy metals involves in the binding of MTF-1 (Langmade, Ravindra, Daniels, & Andrews, 2000). The MT concentration has been demonstrated to play an important role in high capacity to chelate cadmium, and the sequestration and detoxification of various heavy metal ions (Kling & Olsson, 2000; Méndez-Armenta et al.., 2003; Roesijadi, 1994). In present study, it has been proven that LA had significant effects on the gene expression of MT via upregulating the gene expression of MTF-1 in abalone. The capability that MT could chelate Cd in abalone tissues was also shown. The Cd bioaccumulation in serum, muscle, mantle, gill and hepatopancreas was significantly reduced with the increased MT concentration in hepatopancreas in LA-treated groups. The above studies suggested that the activity of MT motivated by LA and regulated by MTF-1 plays an important role in chelating Cd and decreasing its deposition in abalone tissues. However, LA had no significant effects on Cd accumulation in the shell. The possible reason could be that the shell of abalone consists of more than 95% calcium carbonate and less than 5% organic matrix, which is not provided with physiological activity (Blank et al., 2003). Therefore, LA played an important role in soft tissues rather than in shell. In addition, compared with control group, the slightly increased concentration of shell Cd in LA-treated ones may be related to its geochemical properties, the ionic radius of Cd is similar with Ca (cf. Ca 9.7 and Cd 9.8 nm; Huanxin, Lejun, & Presley, 2000). Accordingly, Cd chelated by MT in the soft tissues was partially eliminated, and the others might be involved in shell mineralization as a substitute for Ca. However, further work is absolutely needed to make it clear.

The present study showed the reduced concentration of MDA and protein carbonyl in hepatopancreas of abalone under the waterborne Cd stress along with the supplementation of LA in diets.

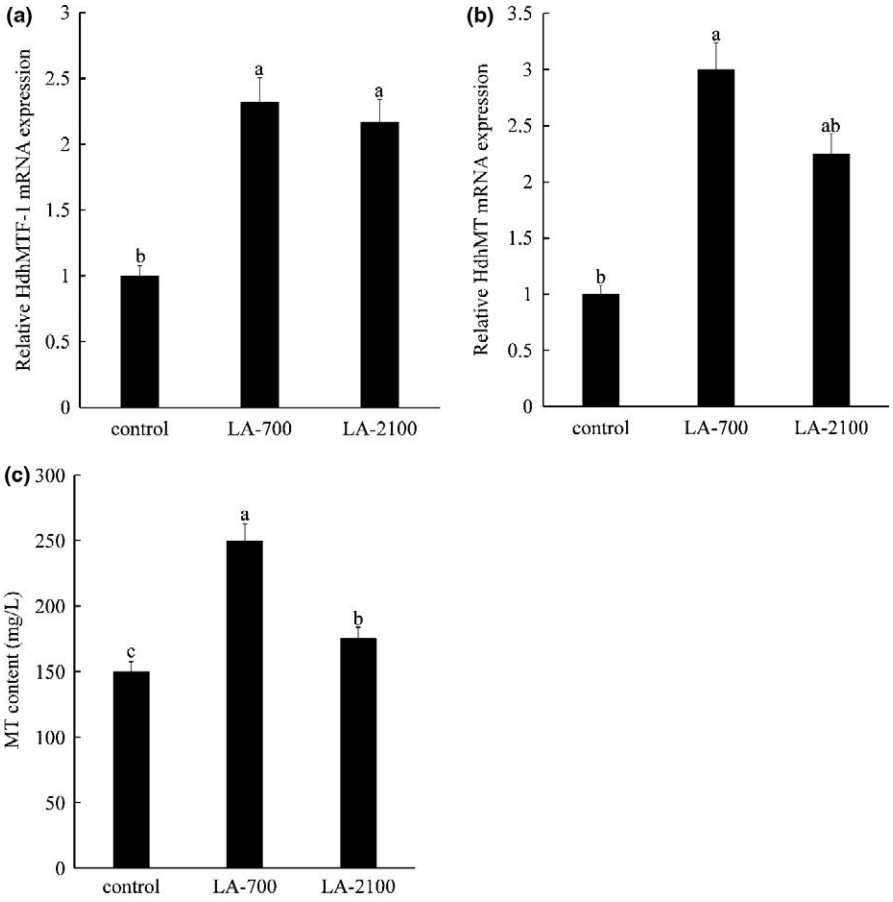

FIGURE 1 HdhMTF-1 mRNA (a) and Relative HdhMT (b) and MT concentration (c) levels in hepatopancreas of abalone *Haliotis discus hannai* fed with dietary LA under waterborne Cd for 2 months respectively. All values were presented as the mean ± *SEM* (n = 3 replicates, and 3 abalones/each replicate). Bars with different letters are significantly different ($p < 0.05$; Tukey's test)

It has also been reported that LA can significantly decrease the level of MDA and protein carbonyls induced by oxidative stress in mice (Manda, Ueno, Moritake, & Anzai, 2007). Oxidative stress in organisms occurs when ROS generation exceeds that of their removal by antioxidative systems (Matés, Segura, Alonso, & Márquez, 2008). ROS cause lipid peroxidation and oxidation of some specific proteins, thus affecting many intra- and intercellular systems (Mates, Perez-Gomez, Olalla, Segura, & Blanca, 2000). MDA and protein carbonyl are known to be acted as biomarker of oxidative stress (Hipkiss, Preston, Himswoth, Worthington, & Abbot, 1997; Pirinccioglu, Gökalp, Pirinccioglu, Kizil, & Kizil, 2010). Previous studies showed that excess Cd bioaccumulation lead to the overproduction of ROS (Chater et al., 2008). In present study, dietary LA significantly reduced the Cd bioaccumulation, thereby could decrease the ROS generation. Combining the results of the enhanced antioxidative responses, it was suggested that LA alleviates Cd-induced oxidative damage through enhancing antioxidative capacities and reducing the Cd accumulation in abalone.

In conclusion, the present study provided evidences that LA supplementation in diet could help abalone against Cd-induced oxidative damages. LA can significantly increase the antioxidative capacities and decrease the Cd bioaccumulation by enhancing the concentration of MT. All of them contributed to the beneficial effects of LA on the decreasing concentration of MDA and protein carbonyl. The present results open a new potentiality in further use of LA as a heavy metal antidote against waterborne Cd in abalone.

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