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ISSN 0792 - 156X

PUBLISHER:
Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

Effects of Selenium on the Activity of Antioxidant Enzymes in the Shrimp, *Neocaridina heteropoda*

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(Received 10.4.09, Accepted 23.5.09)

Key words: *Neocaridina heteropoda*, selenium, antioxidant enzymes

Abstract

The effects of a selenium-supplemented diet on the antioxidant enzyme activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) in muscles of the shrimp, *Neocaridina heteropoda*, were investigated. Purified diets with six levels (0, 0.15, 0.30, 0.45, 0.60, and 0.75 µg/g) of supplemental Se were fed to *N. heteropoda* for three months. CAT and GPX activity was determined after one month and SOD activity was determined every month. The activity of the enzymes was dose dependant; activity of all three enzymes was significantly higher in shrimp fed Se-supplemented diets than in those fed the unsupplemented control diet. SOD activity significantly rose and fell during the three months and was higher in the second month than in the first or third. For all three enzymes, antioxidant activity reached a maximum when the Se concentration was 0.45 µg/g.

Introduction

Significant environmental impacts of aquaculture include industrial effluents, habitat destruction, water pollution, and over-fishing of native parent fishes. Due to the development of intensive cultivation modes, aquaculture has grown rapidly. Frequent feeding, drug and disinfectant abuse, and inappropriate management methods have resulted in the deterioration of aquaculture environments, increased disease among cultured animals, and a serious reduction in shrimp farmers' income. Therefore, to achieve sustainable development of aquaculture, it is important to seek effective methods of alleviating or eliminating environmental stress.

Selenium is an essential trace element for organisms. It has a biochemical role in synthesizing selenoproteins such as glutathione peroxidase (GPX), an antioxidant enzyme. The influence of dietary Se upon the activity of GPX is well documented (Bugel et al., 2001). Animals require

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an appropriate dose of Se, otherwise they display Se-deficiency symptoms or chronic acute toxicities, called the “Se Weinberg principle” or “Se dose-response relationship curve” (Foster and Sumar, 1997). Se plays a positive role when supplemented at the recommended dietary level. However, high amounts of Se display a negative role.

In mammalian phagocytic cells, oxygen-dependent defense mechanisms result in the generation of reactive oxygen species (ROS) with powerful microbiocidal activity (Babior, 1984). Working with separated hemocyte fractions, Bell and Smith (1993) demonstrated the generation of superoxide anions (O_2^-) by hemocytes of the decapod *Carcinus maenas*. Munoz et al. (2000) demonstrated the production of superoxide anions by hemocytes of the white shrimp *Penaeus vannamei*. Environmental contaminants can lead to non-infectious diseases. Ahmad (1995) found evidence for oxidative stress-related pathologies from pollutants in marine organisms. Aquatic organisms are protected against ROS by antioxidant enzymes and low molecular weight scavengers (Winston and Giulio, 1991; Peters and Livingstone, 1996).

So far, studies on the effect of Se on fish have been reported (Hilton et al., 1980; Liang et al., 2006). But there is little information on the effect of Se on crustacean antioxidant enzymes. The purpose of this research was to determine the effects of dietary Se on the antioxidant response of *Neocaridina heteropoda* and the optimum dietary Se requirement for maximum growth and maintenance of non-specific immune responses in this species. Hopefully, our study will provide useful information for improving the tolerance of *N. heteropoda*, a small commercially important species found in fresh water throughout China.

Materials and Methods

Shrimps. *Neocaridina heteropoda* were collected from Baiyangdian Lake in Hebei Province, China, in March 2008 and acclimated in a holding tank for one week. Nine hundred shrimps (mean wt 0.30±0.11 g) were selected for the experiment. They were reared in a freshwater recirculating system in tanks (50 x 50 x 20 cm^3) aerated with aeration stones attached to air pumps and containing fifty shrimps each. Water was replaced at 10% daily. Temperature was kept at 20±1°C using aquarium heaters. The Se concentration in the rearing water was 0.01 mg/l, determined by atomic fluorescence spectrometry (Stibilj et al., 2003).

Diets. The composition of the basal diet included soybean oil, wheat starch, soybean meal, corn starch, vitamin premix, and mineral premix (Table 1). The concentration of unsupplemented selenium in the basal feed (0.005 μg/g) was determined by atomic fluorescence photometry (Dagnall et al., 1969). Na_2SeO_3 was used as the source of supplemental Se. Supplemented diets contained 0.15, 0.30, 0.45, 0.60, and 0.75 μg selenium per g diet. Prepared diets were stored at -20°C in sealed plastic bags.

Feeding. Three replicate tanks were assigned to each treatment. The diets were fed to shrimps in the morning and at night at a daily rate of 5% of their body weight. Feces and excess feed were removed in the morning before feeding. Shrimps were weighed at the beginning and the end of the experiment. After 30 days, shrimps in the intermolt stage as determined by Peebles (1977) were randomly selected for trial of antioxidant enzyme activities. Mortality was measured throughout the experiment.

Antioxidant enzyme activity assays. Abdomen muscles were taken from the shrimps, immediately freeze-dried in liquid nitrogen, and homogenized in nine volumes of 20 mmol/l phosphate buffer of pH 7.4, 1 mmol/l EDTA, and 0.1% triton X-100. The homogenates were centrifuged at 600 x *g* to remove debris, and the resultant supernatants were used immediately for enzyme assay. The protein content of the homogenate was measured with a spectrophotometer using bovine serum albumin as the standard, according to the method of Bradford (1976). Absorbance of the samples was detected at 595 nm.

Total superoxide dismutase (SOD; EC 1.15.1.1) activity was determined using the method of Marklund and Marklund (1974) based on the autooxidation of pyrogallol, modified by Jing and

Table 1. Composition of the basal diet.

Ingredient	*Per kg*
Bean cake	412 g
Soybean oil	31 ml
Wheat starch	227 g
Soybean meal	237 g
Corn starch	52 g
Vitamin premix[a]	21 g
Mineral premix[b]	20 g

[a] Supplied (per kg diet): vitamin A 300,000 IU, riboflavin 480 mg, pyridoxine 360 mg, cyanocobalamin 1.2 mg, thiamin 20.0 mg, menadione 20 mg, folic acid 170 mg, biotin 10 mg, a-tocopherol 3000 IU, myo-inositol 8000 mg, calcium pantothenate 800 mg, nicotinic acid 200 mg, choline chloride 8000 mg, vitamin D 40,000 IU

[b] Supplied (per kg diet): $ZnSO_4 \cdot 7H_2O$ 0.817 g, $CaCO_3$ 3.28 g, NaH_2PO_4 2.96 g, KH_2PO_4 6.752 g, $CaCl_2$ 1.3328 g, $MgSO_4 \cdot 7H_2O$ 1.6 g, KCl 0.448 g, $AlCl_3 \cdot 6H_2O$ 0.0192 g, $MnSO_4 \cdot (4\text{-}6)H_2O$ 0.229 g, $CuCl_2$ 0.52 g, $FeSO_4 \cdot 7H_2O$ 1.8 g, $CoCl_2$ 0.0282 g, KI 0.036 g

Zhao (1995). A reference standard SOD was supplied with the Ransod Kit. One unit of SOD was defined as the amount required to inhibit the rate of xanthine reduction by 50%. Specific activity was expressed as SOD units/mg protein (Biagini et al., 1995).

Glutathione peroxidase (GPX; EC 1.11.1.9) activity was measured by following the rate of NADPH oxidation at 340 nm and by the coupled reaction with glutathione reductase (Bell et al., 1985).

Catalase (CAT; EC 1.11.1.6) activity was measured by an ultraviolet spectrophotometer (Beers and Sizer, 1952). A 10-μl sample was added to 3.0 ml of H_2O_2 phosphate buffer of pH 7.0 (0.16 ml of 30% H_2O_2 to 100 ml of 67 mmol/l phosphate buffer) and the variation of H_2O_2 absorbance in 60 s was measured with a UV-2100 spectrophotometer at 250 nm. One unit of enzyme activity was defined as the amount of enzyme that reduces the concentration of H_2O_2 by 50% in 100 s at 25°C.

Statistical analysis. Results are expressed as means±SD (n = 6) and analyzed using SPSS version 11.5. Differences between mean values were analyzed by one-way analysis of variance followed, when pertinent, by Tukey's post hoc test.

Results

The mean body length and weight of all shrimps fed selenium diets differed from the contol and shrimp fed the 0.45 μg/g diet had significantly (p<0.05) higher values than the others (Table 2). At low levels of Se (less than 0.45 μg/g), total SOD and GPX activities rose slightly and significantly varied (p<0.05) from the control (Fig. 1). Activity of all three enzymes peaked with the 0.45 μg/g treatment. At this concentration, SOD, CAT, and GPX activity were 2.26, 9.27 and 2.35 times, respectively, higher than in the control. Beyond 0.45 μg/g, all three enzymes significantly dropped with the increasing Se concentration. The effects of Se concentration on the SOD activity of *N. heteropoda* were significantly influenced by time; SOD activity was higher at two months than at one or three months (Fig. 2).

Discussion

Effects of dietary selenium on growth performance. After 30 days, Se supplementation caused a significant increase (p<0.05) in specific growth rate compared to the control in all groups. Shrimps fed the 0.45 μg/g diet had the highest values. *Penaeus chinensis* also grew better after Se supplementation (Wang et al., 1994).

Effects of dietary selenium on antioxidant enzyme activities. Environmental variations often induce changes in the immune status of crustaceans. These variations may be stressful for crustaceans and result in a reduction of immune ability, measured by the release of reactive oxygen species (ROS; Moullac and Haffiner, 2000). ROS include the superoxide anion (O_2^-), hydrogen

Table 2. Mean growth rates of body length and body weight of the shrimp, *Neocaridina heteropoda*, fed a selenium-supplemented diet for one month.

Groups	*Se (μg/g)*	*Body length (%)*	*Body weight (%)*
0	0	17.0±6.8	13.1±1.2
1	0.15	32.0±9.1*	24.1±2.3*
2	0.30	35.4±6.2*	28.9±2.7*
3	0.45	41.0±9.7*	30.3±3.3*
4	0.60	30.7±7.7*	23.4±1.7*
5	0.75	25.8±4.4*	23.3±1.3*

* Significantly different from the control at $p<0.05$.

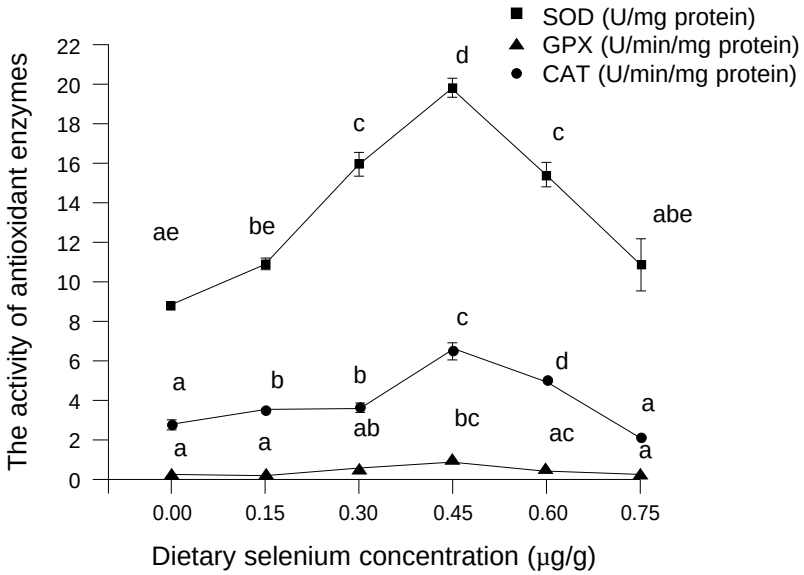

Fig. 1. Antioxidant enzyme activity of superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) in shrimp, *Neocaridina heteropoda*, fed diets with different concentrations of selenium. Significant differences ($p<0.05$) are indicated by different letters (means±S.D., n = 6).

peroxide (H_2O_2), hydroxyl radical (OH), and singlet oxygen ($^1O_2$). The superoxide anion is the first to be released during respiratory burst and plays an important role in microbicidal activity (Bell and Smith, 1993; Munoz et al., 2000). ROS are able to attack almost all bio-molecules in their vicinity causing protein modification, lipid peroxidation (LPX), and DNA strand breakage in cells (Halliwell and Gutteridge, 1989) that may eventually lead to pathological states including apoptosis (Buttke and Sandstorm, 1994).

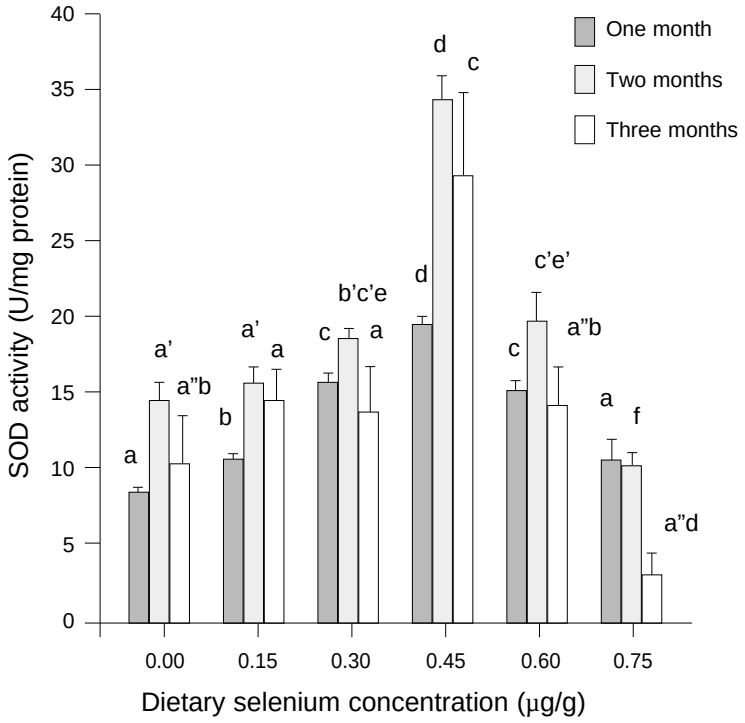

Fig. 2. The effect of dietary selenium on the activity of superoxide dismutase (SOD) in the muscles of the shrimp, *Neocaridina heteropoda*, at the end of each of three months. Significant differences (p<0.05) are indicated by different letters (means±S.D., n = 6).

Under normal physiological states, the harmful effects of ROS are effectively neutralized by an interacting network of antioxidant enzymes (Davies, 1995). The superoxide released by processes such as oxidative phosphorylation is first converted to hydrogen peroxide and then reduced to water. This detoxification pathway is the result of multiple enzymes with SOD catalyzing the first step and then CAT, GPX, and various peroxidases removing hydrogen peroxide (Sies, 1997). The activity of SOD has been measured in the shrimps *Palaemontes argentinus* and *Litopenaeus vannamei* (Neves et al., 2000; Campa-Cordora et al., 2002). In the presence of low H_2O_2 levels, organic peroxides are the preferred substrate for GPX. However, at high H_2O_2 concentrations, they are metabolized by CAT (Yu, 1994). In our study, GPX and CAT activities increased initially and then decreased with the increasing Se concentration. SOD could constitute a good molecular-bioindicator for oxidative stress and acute pollution (Biagini et al., 1995). Since the antioxidant enzymic activities of shrimps fed Se-supplemented diets were significantly higher than those of shrimps fed the basal diet, dietary Se supplementation could enhance the antioxidant enzymic activity of *N. heteropoda.*

Optimum selenium requirements. Selenium deficiency is relatively rare in healthy well-nourished individuals. Salmon may exhibit signs of muscular dystrophy, anemia, and even death if lacking biological Se (Poston et al., 1976; Bell et al., 1986a,b). Although selenium is an essential trace element, it is toxic if taken in excess (Hamilton et al., 2002). The supplementation of

diets with high levels of Se (1.0 µg/g) was harmful and increased nitrite toxicity in prawns (Wang et al., 2004). It is possible that excess Se catalyzes the naissance of active oxygen radicals (Seko and Imura, 1997). In prawn tissue, Se analogues of sulfur-containing enzymes and structural proteins might play a role in toxicity caused by dietary Se (Wang et al., 2006).

The optimum Se requirement was calculated to be 0.15-0.38 µg/g for rainbow trout (Hilton et al., 1980), 0.25 µg/g for fingerling channel catfish (Gatlin and Wilson, 1984), 0.4 µg/g for juvenile Japanese sea bass (Liang et al., 2006), 0.396-0.529 µg/g for juvenile carp (Lin and Shiau, 2005), and 0.44 µg/g for *P. chinensis* (Wang et al., 1994). Proper quantities of dietary Se enhance the immunity of shrimps and improve growth.

Effect of time on SOD activity. There is little information on the correlation between time and the effects of Se on the antioxidant enzyme activities of crustaceans. In this study, the effects of Se concentration on SOD activity were significantly influenced by time (Fig. 2). Whether the accumulation of Se in shrimp tissues represents a potential hazard to the shrimp or to people eating the shrimp needs to be further investigated (Bugel et al., 2001).

Conclusions

The weight gain of shrimp improved as the level of supplemented Se increased, reaching a peak when the concentration of Se was 0.45 µg/g diet. A similar trend was observed in the non-specific immune responses of the shrimp. Se concentration in the muscles increased as the dietary Se level increased until a peak of 0.45 µg/g. In summary, the adequate dietary Se requirement for maximal growth and maintenance of non-specific immune responses in *N. heteropoda* is 0.45 µg/g. Further research is needed to investigate the molecular mechanisms of Se that affect the antioxidant enzyme responses of *N. heteropoda*.

Acknowledgements

We wish to thank Drs. Zhen-Yu Zhang, Yi-Feng Du, and Zhi-Ping Mei for modifying this manuscript. We would also like to express our appreciation to Dr. Jomo MacDermott and Mr. Cornell Lee, foreign English instructors for the Life Science College of Hebei University, for editing this manuscript. We are grateful for the constructive comments of the reviewers. This research was supported by the project Excellent Youth Teacher Domestic Visiting Scholar, Ministry of Education of the People's Republic of China; the Natural Science Foundation of Hebei Province, P.R. China (C2006000767, C2007001015, and C2007000193); Key Subject of Zoology in Hebei province of P.R. China; Project of Ph.D. fund in Hebei University; and Biology of Hebei University, A Superior and Distinguished Discipline of Higher Education, Hebei Province.

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