



## Dietary selenium requirement of juvenile oriental river prawn *Macrobrachium nipponense*



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### ABSTRACT

Selenium (Se) is an essential nutrient for crustaceans. The dietary selenium requirement of juvenile oriental river prawn *Macrobrachium nipponense* was evaluated. Yeast-selenium was added to basal semi-purified diet at six levels to make diets with the concentration of 0.11, 0.31, 0.47, 0.59, 0.88 and 1.17 mg Se kg<sup>-1</sup>. These diets were fed to juvenile prawns (initial weight 0.133 ± 0.003 g) in quintuplicate for 8 weeks. Our results indicated that the growth performance of prawn was significantly affected by dietary selenium. Prawns fed the 0.47 and 0.59 mg Se kg<sup>-1</sup> diets achieved the highest weight gain rate (WGR), whereas the deficiency (0.11 mg kg<sup>-1</sup>) and excess (1.17 mg kg<sup>-1</sup>) of Selenium inhibited their growth. Whole-body and muscle Se concentrations generally increased with the level of dietary Se. The highest concentrations in body and muscle were observed in the prawns fed the 1.17 mg kg<sup>-1</sup> diet, which were significantly higher than those of prawns using 0.11, 0.31, 0.47 and 0.59 mg kg<sup>-1</sup> diets. Selenium also affects the activity of antioxidant enzymes. The activities of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) were significantly higher in prawns fed with 0.31, 0.47 and 0.59 mg Se kg<sup>-1</sup> than other groups, whereas the maleic dialdehyde (MDA) content had the opposite pattern from GSH-Px activity. Significantly elevated glutathione reductase (GR) activity and reduced glutathione (GSH) content were found in prawns fed the 0.31, 0.47, 0.59 and 0.88 mg kg<sup>-1</sup> diets. The prawns fed with 0.31–0.88 mg Se kg<sup>-1</sup> exhibited the highest total antioxidant competence (T-AOC activity), which was significantly higher than 1.17 mg kg<sup>-1</sup> group. Furthermore, selenium is involved in prawn immune competence. Serum phenoloxidase (PO) and lysozyme activities in prawns fed the 0.31, 0.47, 0.59 and 0.88 mg Se kg<sup>-1</sup> diets were significantly higher than those fed the 0.11 and 1.17 mg Se kg<sup>-1</sup> diets. The minimum dietary selenium requirements for maximal growth and antioxidant ability of juvenile oriental river prawn were evaluated as 0.58–0.68 mg kg<sup>-1</sup> diet based on WGR and GSH-Px activity in the hepatopancreas, and 1.07 mg Se kg<sup>-1</sup> diet was required to maximize whole body selenium content.

**Statement of relevance:** In this study, we evaluated the minimum dietary selenium requirement for juvenile oriental river prawn *Macrobrachium nipponense*. Dietary selenium deficiency and excess significantly reduced growth, antioxidant ability and immunity of prawn. Our results illustrate that supplementing appropriate amount of dietary selenium is necessary in prawn culturing. The minimum dietary selenium level for maximal growth and antioxidant ability of juvenile *M. nipponense* is recommended as 0.58–0.68 mg kg<sup>-1</sup> diet based on the analyses of WGR and GSH-Px activity, and 1.07 mg Se kg<sup>-1</sup> diet is required to maximize whole body selenium content. This research will facilitate to the development of practical diet for prawn.

### 1. Introduction

Selenium (Se) is a fundamental nutrient for animals including crustaceans (National Research Council, 2011; Watanabe et al., 1997). Physiologically, selenium is an integral component of glutathione peroxidase (GSH-Px) in the form of selenocysteine (Rotruck et al., 1973). This enzyme protects cell membranes against oxidative

damage by using reduced glutathione to catalyze the reactions necessary for conversion of hydrogen peroxide and fatty acid hydroperoxides into water and fatty acid alcohol (Dröge, 2002; Lin and Shiau, 2005). In general, selenium affects the growth, development and health of organisms directly (Shelby and Popham, 2007; Marin-Guzman et al., 2000; Sritunyalucksana et al., 2011). Dietary Se requirements have been reported in several fish species (Liu et al., 2010; Zhu et al., 2012;

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Hilton et al., 1980; Gatlin and Wilson, 1984; Liang et al., 2006; Su et al., 2007). However, information for Se requirements in crustaceans is limited, with studies only on Chinese mitten crabs (*Eriocheir sinensis*) (0.40–0.60 mg kg<sup>-1</sup>) (Tian et al., 2014) and shrimp (*Litopenaeus vannamei*) (0.20–0.40 mg kg<sup>-1</sup>) (Davis, 1990).

Although aquatic organisms can directly uptake selenium from water, acquisition of selenium from food is the dominant mode (Watanabe et al., 1997). Dietary selenium deficiency was associated with high mortality and immunosuppressive effects in Yellowtail Kingfish (*Seriola lalandi*) (Le and Fotedar, 2014a, b), giant freshwater prawn (*Macrobrachium rosenbergii*) (Chiu et al., 2010) and marron (*Cherax cainii*) (Nugroho and Fotedar, 2013). Conversely, high dietary selenium might be toxic to aquatic animals. The toxicity of excess dietary selenium to abalone (*Haliotis discus hannai*) was characterized by depressed growth performance and decreased phenoloxidase (PO) and lysozyme activities in serum (Wang et al., 2012). Rainbow trout (*Oncorhynchus mykiss*) fed diets with high selenium exhibited reduced growth rates, poor feed efficiencies and high mortality rates (Hilton et al., 1980). Selenium has a narrow margin of tolerance in biological systems (Maier and Knight, 1994). Thus, evaluating the optimal dietary selenium requirement is very important for the healthy growth and culture of aquatic organism.

Until now, the organic source of yeast selenium and selenomethionine is recognized as the more efficient as feed additives. Compared with inorganic selenium of selenite, organic selenium is absorbed at a higher rate, has greater bioavailability and is better retained in the organism (Lin, 2014; Wang and Lovell, 1997; Küçükbay et al., 2009; Bell and Cowey, 1989; Le and Fotedar, 2014a). The use of organic selenium as a dietary supplement has been reported in yellowtail kingfish (Le and Fotedar, 2014a, b), rainbow trout (Amirkolaie et al., 2014; Vidal et al., 2005), marron (Nugroho and Fotedar, 2013) and other aquacultured species. In this study, the organic selenium source of yeast selenium was employed.

Oriental river prawn (*Macrobrachium nipponense*) is a traditional and popular aquaculture species that has been widely farmed in China, Japan and Southeast Asian countries. In 2015, the production of farmed *M. nipponense* in China was  $2.65 \times 10^5$  t, increased by 2.88% than in 2014 (Ministry of Agriculture Fisheries Bureau, 2016). The rapid development of *M. nipponense* cultivation raises the need of determining this species' dietary requirements. To date, the nutritional requirements of *M. nipponense* for protein (Yu and Shen, 1990; Zhang et al., 2008; Yang et al., 2004), lipid (Yu and Shen, 1990), copper (Kong et al., 2014) and zinc (Guo et al., 2013) have been studied. However, very little information about dietary selenium requirement for prawn has been evaluated. Therefore, we aimed to quantify the dietary Se requirement of juvenile *M. nipponense* and to investigate the effects of dietary selenium level on antioxidant activities and immunity of prawn.

## 2. Materials and methods

### 2.1. Diets preparation

The semi-purified basal diet was formulated using casein and fish meal as protein sources, fish oil and soybean oil as lipid sources (Table 1). Yeast-selenium (Angel Yeast CO., LTD, Yichang, China) was added to a basal diet at 0.00, 0.20, 0.40, 0.60, 0.80 and 1.20 mg Se kg<sup>-1</sup> dry diet with corresponding decreases in the amount of cellulose. Process of diet preparation was similar to that described by Li et al. (2010). Selenium concentrations in the diets were analyzed by hydride generation atomic absorption spectrophotometry (Tinggi, 1999) to be 0.11, 0.31, 0.47, 0.59, 0.88 and 1.17 mg kg<sup>-1</sup> respectively. The dry pellets were sealed in plastic bags and stored at -20 °C until use. The diet formulation and composition analysis of the basal diet are given in Table 1.

**Table 1**  
Ingredients and approximate compositions of the basal diet (% dry weight).

Ingredient	%
Casein <sup>a</sup>	30
Fish meal <sup>b</sup>	20
Corn starch	26
Fish oil <sup>b</sup>	4
Soybean oil <sup>c</sup>	2
Vitamin mix <sup>d</sup>	2
Se-free mineral mix <sup>e</sup>	3
Attractant <sup>f</sup>	3
Cholesterol <sup>g</sup>	0.5
Choline chloride <sup>g</sup>	0.5
Lecithin <sup>g</sup>	0.5
Cellulose	6.5
Sodium carboxymethylcellulose <sup>g</sup>	2
Proximate composition	
Crude protein	41.32
Crude lipid	8.03
Crude ash	7.46

<sup>a</sup> Sigma-Aldrich Co., Shanghai, China.

<sup>b</sup> Qingdao Great Seven Bio-Tech Co. Ltd., Qingdao, China.

<sup>c</sup> National Golden Dragon Fish Co. Ltd, Shanghai, China.

<sup>d</sup> Vitamin mixture (mg 100 g<sup>-1</sup> mixture): vitamin A 420000 IU, vitamin C 6000 mg,  $\alpha$ -tocopherol acetate 2000 mg, vitamin D3 120,000 IU, vitamin K 1000 mg, vitamin B1 1000 mg, vitamin B2 1000 mg, vitamin B6 1600 mg, vitamin B12 2 mg, niacin 5000 mg, folic acid 400 mg, inositol 6000 mg, biotin 10 mg, calcium pantothenic 3500 mg, Hangzhou Minsheng Bio-Tech Co., Ltd., China.

<sup>e</sup> Composition of mineral mixture (g kg<sup>-1</sup> diet): KCl 0.84, MgSO<sub>4</sub>·7H<sub>2</sub>O 3, NaH<sub>2</sub>PO<sub>4</sub> 6.45, KH<sub>2</sub>PO<sub>4</sub> 3, Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>·H<sub>2</sub>O 7.95, CaCO<sub>3</sub> 3.15, C<sub>6</sub>H<sub>10</sub>CaO<sub>6</sub>·5H<sub>2</sub>O 4.95, FeC<sub>6</sub>H<sub>5</sub>O<sub>7</sub>·5H<sub>2</sub>O 0.36, CuSO<sub>4</sub>·5H<sub>2</sub>O 0.1055, ZnSO<sub>4</sub>·7H<sub>2</sub>O 0.1428, MnSO<sub>4</sub>·H<sub>2</sub>O 0.0321, AlCl<sub>3</sub>·6H<sub>2</sub>O 0.0045, CoCl<sub>2</sub>·6H<sub>2</sub>O 0.042, KI 0.0069.

<sup>f</sup> Alanine 0.6%, glycine 0.6%, glutamic acid 0.6%, betaine 1.2%.

<sup>g</sup> China National Medicine Corporation Co., Ltd, Beijing, China.

### 2.2. Experimental animals and feeding trials

All juvenile prawns were obtained from a local farmer (Huzhou, China). Prior to the experiment, prawns were acclimatized to the laboratory conditions for 2 weeks. At the beginning of the experiment, healthy prawns with an average initial weight of  $0.133 \pm 0.003$  g randomly stocked in thirty 300 L tanks with 50 prawns per tank (five replicates per dietary group). The feeding trial was conducted from June to August 2014. Prawns were fed ad libitum twice a day (08:00 and 17:00 h). One third of the tank water was exchanged daily to maintain water quality. The temperature of water ranged from 27 °C to 31 °C, dissolved oxygen was maintained above 6.5 mg L<sup>-1</sup> with ammonia and nitrate below 0.1 mg L<sup>-1</sup> during the feeding trial. Prawns were exposed to a natural photoperiod and fed to for 8 weeks. The selenium concentration in rearing water was also monitored every two weeks.

### 2.3. Sample collection

At the end of the feeding trial, the prawns were not fed 24 h before sampling. Six prawns from each tank were randomly collected and stored at -20 °C for the measurement of Se concentration. Hemolymph was withdrawn using nonheparinized syringes from the ventral sinus of the remaining prawns in each tank and clot at 4 °C overnight. Serum samples were collected following centrifugation and stored at -20 °C for PO and lysozyme activities analyses. Then muscle and hepatopancreas were dissected and stored at -80 °C. The contents of maleic dialdehyde (MDA) and reduced glutathione (GSH), activities of total antioxidant competence (T-AOC), superoxide dismutase (SOD), glutathione reductase (GR) and GSH-Px of hepatopancreas were determined.

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