Fish & Shellfish Immunology 54 (2016) 481–488

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Dietary nano-selenium relieves hypoxia stress and, improves immunity and disease resistance in the Chinese mitten crab (*Eriocheir sinensis*)

Fenju Qin [a, *], Miaomiao Shi [b], Hongxia Yuan [a], Linxi Yuan [c, f], Wenhao Lu [d], Jie Zhang [e], Jian Tong [e], Xuehong Song [b, **]

[a] Department of Biological Science and Technology, Suzhou University of Science and Technology, Suzhou 215009, China
[b] School of Biology and Basic Medical Sciences, Soochow University, Suzhou 215123, China
[c] School of Earth and Space Sciences, University of Science and Technology of China, Hefei 230026, China
[d] Taicang Fishery Guidance Station, Suzhou 215400, China
[e] School of Public Health, Medical College of Soochow University, Suzhou 215123, China
[f] Jiangsu Bio-Engineering Research Centre of Selenium, Suzhou 215123, China

ARTICLE INFO

Article history:
Received 27 January 2016
Received in revised form
22 April 2016
Accepted 29 April 2016
Available online 3 May 2016

Keywords:
NanoSe
Hypoxia
Immunity
Hemocytes
Antioxidant capacity
Hemocyanin

ABSTRACT

Hypoxia is a relevant physiological challenge for crab culture, and the hemolymph plays a crucial role in response to the hypoxia. In a 60 d feeding trial, Chinese mitten crabs (*Eriocheir sinensis*) fed a diet containing 0.2 mg/kg nano-selenium (nanoSe) showed a significantly increased weight gain rate (WGR) and a reduced feed coefficient (FC) compared to those fed diets with 0, 0.1, 0.4, 0.8, and 1.6 mg/kg nanoSe. Another 90 d feeding trial was conducted to determine the influence of dietary nanoSe on the immune response in juvenile Chinese mitten crabs kept under the condition of hypoxia. The results showed that hypoxia stress resulted in significantly increased hemocyte counts (THC, LGC, SGC, and HC), expression levels of the hemocyanin gene and protein, lactic acid level, and antioxidant capacity (T-AOC activities, SOD activities, GSH-Px and GSH content) in hemolymph supernatant. When these crabs were infected with *Aeromonas hydrophila* bacteria, hypoxia exposure increased mortality, but it was alleviated by a diet supplemented with 0.2 mg/kg nanoSe. The up-regulative effects of nanoSe (0.2 mg/kg) on antioxidant capacity, hemocyte counts, and hemocyanin expression under hypoxia exposure were further strengthened throughout, whereas lactic acid levels induced by hypoxia stress were restored. Thus, the observations in this study indicate that the level of dietary nanoSe is important in regulating immunity and disease resistance in crabs kept under hypoxia stress.

1. Introduction

With rapid human population growth and global warming, hypoxia has been responsible for serious decline in the quality of the aquatic habitat, which could become worse in the future [1,2]. In recent years, the duration and frequency of hypoxia as well as its severity have increased, and these changes have affected the growth of aquatic organisms and even resulted in death [3–7]. Chinese mitten crabs (*Eriocheir sinensis*) usually are cultured in a pond. The dissolved oxygen (DO) concentration in ponds changes significantly through the night and day, and these changes may have notable effects on crustacean life, particularly crabs reared in ponds without aerators during the hot summer. Water at the bottom of ponds, where crabs live most of their life, may become hypoxic at night due to the organisms' respiration and to decomposition of accumulated organic matter (e.g., feces and uneaten feed). Such hypoxic conditions can certainly threaten the survival of crabs. Hence, hypoxic stress is an important detrimental environmental factor [8] at present in the crab culture industry in China.

Numerous reports of the effects of hypoxia and/or anoxia on vertebrates exist [9,10]. Comparatively less is known about hypoxic effects on the physiology of invertebrates [11]. In aquaculture, most existing research of the impact of hypoxia on crustaceans focused

* Corresponding author.
** Corresponding author.
E-mail addresses: qinfenju@mail.usts.edu.cn (F. Qin), songxh@suda.edu.cn (X. Song).

http://dx.doi.org/10.1016/j.fsi.2016.04.131
1050-4648/

on shrimp and crayfish. Lower DO concentration was found to have a negative effect on the immune system [12,13], leading to decreased resistance to diseases [14,15]. Previous studies of the effects of DO concentration on crabs focused mainly on behavior [16–18], LC50, feeding [19], and respiratory and cardiovascular systems [20,21]. However, little is known about the effect of hypoxia on the immune systems, especially on the hemocyanin gene and protein, in crabs. Moreover, the protection against hypoxia stress in crabs that might be provided by dietary selenium (Se) has not been reported.

Se is a dietary trace element that is essential for humans and animals. It plays an important role in antioxidant defense systems and is a central component of the enzyme glutathione peroxide (GSH-Px) [22]. Se has received a great deal of attention because it is essential for normal functioning of selenoproteins (selenocysteine and selenomethionine) [23], which are involved in numerous biological functions (e.g., preventing oxidative damage, maintaining homeostasis of thyroid hormone, and enhancing immune functions) [24,25]. The micronutrient Se in the diet is essential for normal immune functions and is reported to possess immunoenhancing properties [26]. Appropriate Se intake is necessary, but it has a narrow therapeutic impact [27–29]. Elemental nanoparticles of Se (nanoSe) reportedly are less toxic than inorganic Se compounds [30], but whether nanoSe protects crabs from hypoxia through a hemocyanin-mediated pathway is not known. Therefore, the purpose of the current investigation was to evaluate the effect of supplementing the Chinese mitten crab diet with nanoSe and to determine the optimum concentration to improve disease resistance, immune function, and the hemocyanin gene expression and protein level in the hemolymph of these crabs.

2. Materials and methods

2.1. Preparation of basal diet and test diet

All ingredients of the basal diet and test diet (excluding nanoSe) were supplied by Suzhou Xinyu Feeds Co., Ltd (Suzhou, China). NanoSe (Size: 200–250 nm) was obtained from Jiangsu Bio-Engineering Research Centre of Selenium (Suzhou, China). Table 1 lists the contents of the basal diet. To create the test diets, nanoSe was individually incorporated into the basal diet at levels of 0.1, 0.2, 0.4, 0.8, and 1.6 mg/kg, and the diet without nanoSe addition was used as the control. Crabs diet pellets (2 mm diameter, 3 mm length) were prepared by mixing all ingredients evenly, pelletizing them using a mincer, and drying them in a drying cabinet with an air blower at 40 °C to a moisture level of 10%. After drying, the pellets were allowed to cool to room temperature, and then they were packed and stored at −20 °C.

2.2. Animals and experimental setup

Juvenile Chinese mitten crabs weighing 4.5 ± 0.4 g (wet weight) were obtained from Linhu Lake Modern Fishery Development Co. Ltd. (Suzhou, China). They were immediately transferred to the aquatic laboratory of Soochow University and held in a glass tank (50 × 60 × 100 cm). During the acclimation culture period, each tank was supplied with pre-aerated municipal water at 25 ± 1 °C, pH 7.95 ± 0.06, DO concentration 6.4 ± 0.2 mg/L, salinity 0.3%, total ammonia 0.35 ± 0.02 mg/L, chloride level 135 ± 16 mg/L, and basal nitrite <0.05 mg/L^{-1} with a photoperiod of 12 h light and 12 h dark. The crabs were fed with the basal pellet diet twice daily at a ration of 3% body weight. The water exchange rate was at 20% per day and fecal matter was removed every day.

In experiment 1, 120 crabs were randomly assigned to six different groups and fed with the test diets supplemented with six levels of nanoSe (0, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/kg) for 60 d. Their body weights were recorded every other day. At the end of the experiment, the growth performance index (weight gain rate, WGR) and feed coefficient (FC) were determined, and the optimal level of nanoSe supplementation was determined.

In experiment 2, whether dietary nanoSe improves recovery from hypoxic stress, immunity, and resistance to bacterial infections was tested. In this experiment, 540 crabs were randomly assigned to two groups of 270 crabs. The control group received 0 mg/kg nanoSe, whereas the experimental group received nanoSe at 0.2 mg/kg (based on the optimal WGR and FC levels observed in experiment 1). At the end of 90 d, 90 crabs from each group were divided equally into three sub-groups of 30 crabs each and exposed to hypoxic conditions for 0, 12, or 24 h, respectively. Hemolymph then was collected from these crabs for hemocyte counts (total hemocyte count, THC; granulocyte, GC; semi-granulocyte, SGC; hyalinocyte, HC) and measurement of enzyme activities (total-antioxidant capacity (T-AOC), superoxide dismutase (SOD), glutathione (GSH), GSH-Px, and lactic acid (LA)). The expression levels of the hemocyanin gene and protein were also determined.

The other 180 crabs in the test and control groups were divided equally into three sub-groups of 60 crabs each. They were exposed to hypoxic conditions for 0, 12, or 24 h, respectively. Crabs in each group were injected with 4 × 10^6 CFU/kg *Aeromonas hydrophila* (*A. hydrophila*, CL99920) bacterial suspension through the basipodite of the third pereiopoda and then exposed to hypoxia for the designated amount of time. All crabs in these three sub-groups were used to test resistance to bacterial infection by measuring the immune protection markers. Immune protection efficiency was calculated at each hypoxia time point as [control mortality − nanoSe mortality] x 100/control mortality. Each test was conducted in triplicate, and each replicate contained 20 crabs.

Table 1
Composition of the basal diet (g/kg).

Ingredient	Content	Ingredient	Content
Fish meal	170	Blood meal	30
Cotton seed meal	170	Shrimp shell meal	30
Rape seed meal	160	Soybean oil + rape seed oil	20
Wheat meal	105	$Ca(H_2PO_4)_2$	15
Soybean meal	100	Zeolite powder	20
Corn	90	Crab additive	10
Rice bran	50	Vitamin mix (a)	10
Adhesive	10	Vitamin mix (b)	10

2.3. Hypoxia exposure

Hypoxia was induced using a modified intermittent flow-through system [31]. It consisted of three hermetic tanks: a service tank, a test tank, and a filter tank. The volume in the service and filter tanks was 192 L and that in the test tank was 480 L. Each test tank had 30 compartments, one for each of 30 crabs, and this arrangement was used to prevent cannibalism during the experimental period. The tanks were connected with a soft pipe through which the water from the service tank could flow into the test tank at 60 L/h. The desired level of DO concentration (2.0 ± 0.2 mg/L; hypoxia for the treatment group) in the test tank was regulated using water at 2.0 ± 0.2 mg/L DO from the service tank; this level was established by bubbling nitrogen and air into the water. The crabs exposed to hypoxic water developed hypocapnia. During the experimental period, the DO level was monitored with a DO meter (AM39 model, WTW, Weilheim, Germany) every hour.