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Effect of nitrite exposure on metabolic response in the freshwater prawn *Macrobrachium nipponense*

Research Article

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Abstract: The metabolic response of the freshwater prawn, *Macrobrachium nipponense* to nitrite toxicity was evaluated. The prawns were exposed to 0, 1, 2, 3 and 4 mg L^{-1} NO_2-N concentrations for 48 h. The metabolic parameters in muscle were measured after 12, 24 and 48 h. Glucose level significantly increased after 24 h. Exposure to lower nitrite concentrations (1 and 2 mg L^{-1}) resulted in significant increases in alanine aminotransferase (ALT) activities after 24 and 48 h. Aspartate aminotransferase (AST) activities treated with 2 and 3 mg L^{-1} nitrite-N at 48 h were significantly higher than those at 12 and 24 h. Intermediate sublethal nitrite concentrations produced significant elevations in lactate dehydrogenase (LDH) activities from 12 h up to 48 h. No significant changes were detected in any of the groups for triglycerides and creatine kinase (CK). To satisfy the increased energy demands caused by acute nitrite exposure, mobilization of lipids is not the main reason while utilization of amino acids seems to play a more important role. The results would be helpful for aquaculture farmers to prevent a potential depression of productivity caused by elevated nitrite levels.

Keywords: *Metabolic response • Macrobrachium nipponense*

1. Introduction

Water pollution and industrial waste usually elevate the nitrite concentrations due to ammonia oxidation [1,2]. Nitrite can easily accumulate to an unsafe level and become a serious problem in aquatic ecosystems such as recirculation systems, aquaria and aquaculture ponds [3,4]. In some eutrophic shallow lakes, an elevated concentration of nitrite usually occurs during the degradation of cyanobacterial blooms [5].

The toxicity of nitrite starts from the fact that nitrite is a competitive inhibitor of chloride uptake which can decrease extracellular and intracellular chloride and result in a serious electrolyte imbalance [6]. In crustaceans, the nitrite toxicity has been mainly tested on penaeids with a general understanding that the induction of methaemocyanin by nitrite can cause hypoxia in tissues and impair the respiratory metabolism [7,8]. Additionally, nitrite can cause environmental stress on aquatic animals, and thus retard growth, damage various organs, decrease the tolerance to bacterial or parasitic diseases and even cause high mortality [9,10].

The majority of these studies deal with the nitrite effect on ion exchange, nitrogenous excretion, immune response, *etc*., but very few deal with the metabolic response caused by nitrite. In crayfish, Hemolymph

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glucose levels increased following nitrite exposure independent of nitrite concentrations [11]. In crab, Hong, *et al*. [12] reported that elevation of glucose, glycogenolysis and lactate was induced due to increase of energy demand and anaerobic metabolism under hypoxia in tissue caused by nitrite exposure. In prawns, the metabolic response after nitrite exposure still remains unknown. Furthermore, changes in metabolic variables such as glucose, and triglycerides, which are the main sources of energy, have also been shown to be good indicators of stress in crustaceans [13,14]. Therefore, in this study we choose the freshwater prawn, *Macrobrachium nipponense* which is a commercially important species found in brackish and freshwaters throughout China (from North China to Taiwan), Japan and Vietnam [15,16] as a test species so that we may better understand the acute and sub-lethal effects of nitrite on metabolic response in prawns.

2. Experimental Procedures

2.1 Test organism and rearing conditions

Juvenile *M. nipponense* were obtained from Nanjing Fisheries Research Institute, China, and transported to the aquatic laboratory of Nanjing Normal University. All prawns were held in an indoor recirculating aquaculture freshwater system (Dalian Huixin Titanium Equipment Development Co., Ltd.), at 26±1ºC, pH 7.2, a dissolved oxygen concentration above 5.0 mg L^{-1} by continuously aerating over a 12-h light/dark photoperiod. The prawns were fed with a commercial pellet diet twice daily at a ratio of 4% body weights.

2.2 Nitrite exposure and sample collection

After acclimatized for 2 weeks, 90 prawns (mean weight 1±0.2 g) at the intermolt stage were selected randomly. Because chloride level can strongly affect nitrite toxicity and tap water usually has a higher chloride level than most of the freshwater ponds and lakes, here we use distilled water which was added sodium chloride to get a chloride level of 10 mg L^{-1}. All prawns were held in plastic tanks at 26±1ºC, pH 7.0, a dissolved oxygen concentration about 5.0 mg L^{-1} and a 12-h light/dark photoperiod. Prior to the test feeding was terminated at 24 h and no food was supplied to prawns during the test. Nitrite was added as sodium nitrite to give the required concentration. Based on the 96 h LC50 value [16], five levels of nitrite nitrogen (0, 1, 2, 3, 4 mg L^{-1}) were used for the 48-h acute toxicity test. These assayed values of nitrite are potentially achieved under prawn culture conditions [7]. Six individuals were used per glass tank in three replicates. The test solutions in each glass tank were refreshed every 24 h.

At 12, 24, and 48 h, three prawns for each group were sampled and anaesthetized with ice. The muscles of an individual prawn was removed and rinsed with ice-cold physiological saline with the following composition [17]: NaCl (205 mmol L^{-1}); KCl (5.3 mmol L^{-1}); $CaCl_2$ (13.5 mmol L^{-1}); $MgCl_2$ (2.45 mmol L^{-1}); HEPES (5 m mol L^{-1}) (pH 7.4) About 0.1 g of muscle tissue was homogenized after adding physiological saline solution (muscle/total is 10%), and half of the homogenate was centrifuged at 860*x*g for 10 min at 4ºC. The supernatant was used for activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine kinase (CK) and protein level assays. The other half of the homogenate was centrifuged at 9560*x*g for 10 min at 4ºC and the supernatant was used for glucose and triglycerides assays.

Glucose (mmol g^{-1}), triglycerides (mmol g^{-1}), ALT (U mg^{-1} protein), AST (U mg^{-1} protein), LDH (U mg^{-1} protein) and CK (mg g-1 protein) in muscle of *M. nipponense* were determined using the Diagnostic Reagent Kit purchased from Nanjing Jiancheng Bioengineering Institute (China). Generally, glucose and triglycerides were determined by glucose oxidase-peroxidase-4-aminoantipyrene-phenol (GOD-PAP) and glycerol phosphate oxidase-*p*-aminophenazone (GPO-PAP) methods. Alanine and aspartate were used as the substrates of ALT and AST, respectively. For the measurement of each enzyme activity, relevant substrate, ketoglutarate and assayed sample were incubated at 37°C. The reaction was stopped with 2,4-dinitrophenyl hydrazine. The substance was colored with sodium hydroxide and spectrophotometrically assayed at 505 nm. LDH was assayed using 0.1 mol L^{-1} phosphate buffer (pH 7.5), 0.3 mmol L^{-1} NADH solution in 0.1 mol L^{-1} phosphate buffer solution. 2 mmol L^{-1} of sodium pyruvate was used as the substrate and optical density (OD) was measured at 340 nm. CK was determined according to Tanzer and Gilvarg [18]. Protein content was determined according to Bradford [19] using bovine serum albumin as the standard.

2.3 Statistical analysis

All data were presented as mean ± SE. The data on activities of glucose, triglycerides, AST, ALT, LDH and CK collected at 12 h, 24 h, and 48 h were evaluated by two-way (time and nitrite-N concentration) analysis of variance (ANOVA) followed by Duncan's multiple range test (α=0.05). All statistical analyses were carried out with SigmaPlot 11.0 (Systat Software Inc., Chicago, Illinois).

3. Results

Two-way ANOVA showed that there was a significant effect of nitrite on glucose level in muscle of *M. nipponense* ($P<0.05$). After 12 h of nitrite exposure, the glucose level in 1, 2 and 3 mg L^{-1} groups significantly increased ($P<0.05$). After 24 h of exposure time, in the 1 mg L^{-1} group it returned to the same level as the control group, while it was still significantly higher than the control group in 2 and 3 mg L^{-1} groups ($P<0.05$). But as time elapsed, no significant difference could be found in any of the treatments at 48 h (Figure 1). For muscle triglycerides, no significant changes were detected in any of the groups following different concentrations of nitrite exposure throughout the experimental time (Figure 2).

There were significant effects of both nitrite concentration and exposure time on ALT activity ($P<0.05$), but no significant interaction between nitrite and exposure time was observed. Exposure to lower nitrite concentrations (1 and 2 mg L^{-1}) resulted in significant increases in ALT activities in the *M. nipponense* muscle after 24 and 48 h (Figure 3). No signficant difference of AST activity was observed following nitrite exposure from 12 to 48 h. However, a statistically significant effect of exposure time on AST was detected ($P<0.05$). The values of AST treated with 2 and 3 mg L^{-1} nitrite at 48 h were significantly higher than those at 12 and 24 h ($P<0.05$, Figure 4).

A significant effect of nitrite concentration on muscle LDH activity was found in the study ($P<0.05$). LDH signficantly increased in 2 and 4 mg L^{-1} groups compared to the control at 12 h ($P<0.05$). At 24 h, those exposed to 2 and 3 mg L^{-1} groups was significantly higher than the control group ($P<0.05$). At 48 h, significant increases were observed in the groups exposed to 1 and 2 mg L^{-1}

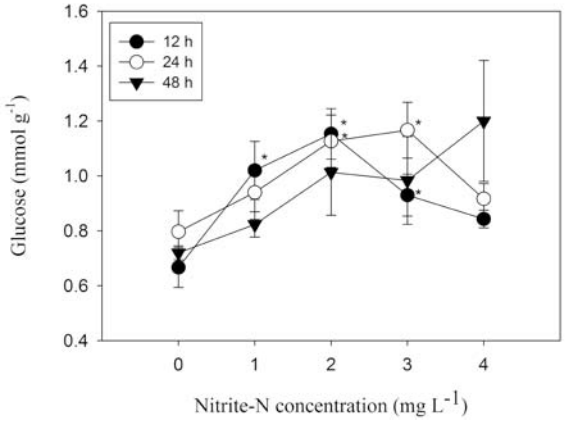

Figure 1. Effect of nitrite exposure on glucose level in the muscle of *M. nipponense.*

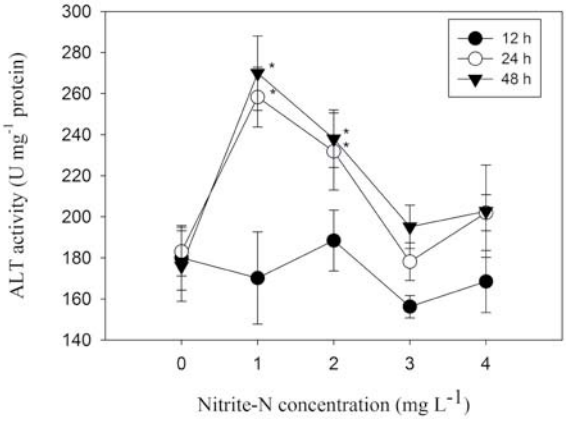

Figure 3. Effect of nitrite exposure on ALT activity in the muscle of *M. nipponense*. Significantly different from controls: *$P<0.05$.

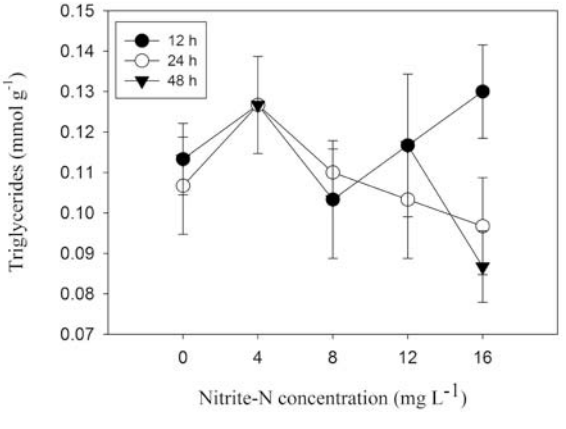

Figure 2. Effect of nitrite exposure on triglycerides level in the muscle of *M. nipponense*. Significantly different from controls: *$P<0.05$.

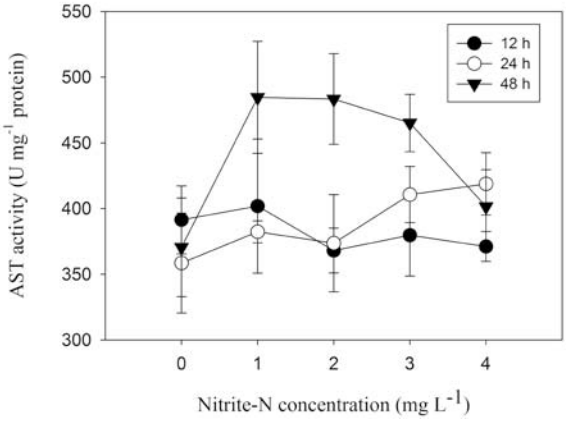

Figure 4. Effect of nitrite exposure on AST activity in the muscle of *M. nipponense*. Significantly different from controls: *$P<0.05$.

(P<0.05, Figure 5). No significant differences of CK activities in the *M. nipponense* muscle were found when measured in any of the groups (Figure 6).

4. Discussion

Coping with stressful conditions depends upon an animal's ability to provide enough fuel to the tissues in order to deal with the allostatic load [20]. Elevated glucose levels in animals was widely used as a secondary marker of responses to stresses such as physical handling [21], emersion or hypoxia [22], de-clawing [23], and high ammonia [24]. It appears that muscle glucose level increased in the present study as a response to stress due to high nitrite exposure, which is in agreement with the effect of nitrite to the freshwater crayfish, *Astacus leptodactylus* [11]. Mobilization of lipid reserves as triglycerides, undergone by the animals testifies high-energy demands [25]. The change in triglycerides was not significant enough to take into account in the current study. It might suggest that mobilization of lipids was not the main reason for the increased energy demand caused by acute nitrite exposure in *M. nipponense*.

ALT and AST are enzymes involved in the transfer of amino groups from one specific amino acid to another. As with other animals, both ALT and AST are key enzymes that allow the interconversion of amino acids and other intermediary metabolites in crustaceans and have been detected in the hepatopancreas, muscle and gill [26]. It is reported that ammonia exposure could increase the activities of ALT and AST in *Nile tilapia* brain [27]. In the present study, the increase of ALT and AST might suggest an increased utilization of amino acids that provides some important substrates allowing energy metabolism to meet the increasing energy demand that occurred during nitrite induced stress [28]. The observation on ALT predominating AST where the feeding of amino acids into energy cycle was mainly through the alanine-pyruvate pathway, suggested that there is an anaerobic tendency of muscle under nitrite stress. Further research is required to investigate protein metabolism in prawns after nitrite exposure.

Exposure to nitrite was reported to cause anoxia in tissues due to the conversion of oxygen-carrying pigments to forms that are incapable of carrying oxygen in fish and crustaceans [10,29]. Lactate dehydrogenase (LDH) is the terminal enzyme of glycolytic pathway, responsible for reversible conversion of pyruvate to lactate and is expected to increase under conditions of stress [30]. The potential of this enzyme as an indicative criterion in invertebrate aquatic toxicity tests has

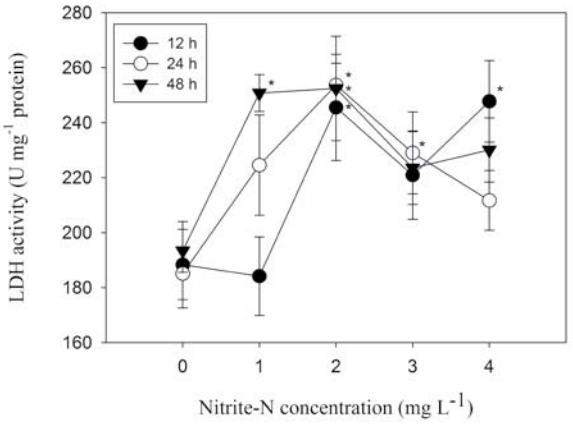

Figure 5. Effect of nitrite exposure on LDH activity in the muscle of *M. nipponense*. Significantly different from controls: *$P<0.05$.

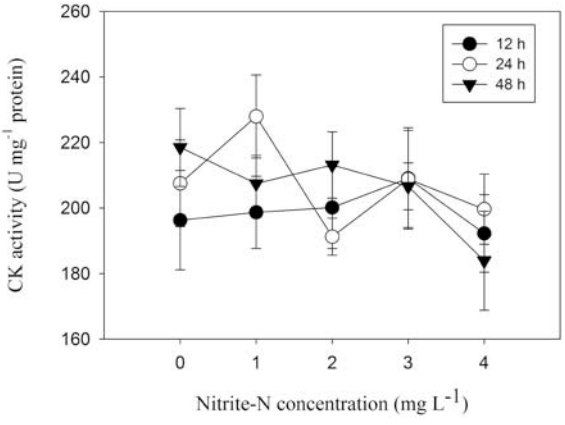

Figure 6. Effect of nitrite exposure on CK activity in the muscle of *M. nipponense*. Significantly different from controls: *$P<0.05$.

been explored [31]. Similarly, Das *et al*. reported that elevations of LDH activities were observed in different tissues of Indian major carps after nitrite exposure [32]. It indicated that hypoxia in tissues caused by nitrite could shift the aerobiosis respiratory metabolism to anaerobiosis to supply more energy for respiratory metabolism [12]. CK catalyses the conversion of creatine and consumes adenosine triphosphate (ATP) to create phosphocreatine and adenosine diphosphate (ADP) and it acts not only acts as an energy buffer but also as a metabolic regulator [33]. It seems that the CK activities in the muscle may not be considered an indicator for nitrite toxicity, since no significant variation was observed in this study.

In conclusion, glucose level increased in the present study as a response to stress due to high nitrite

exposure. For satisfying increased energy demand caused by acute nitrite exposure, mobilization of lipids is not the main reason while utilization of amino acids might play a more important role when nitrite stress occurs. Among all the parameters used in this study, it seems that triglycerides and CK activity in muscle may not be considered good indicators for acute nitrite toxicity in the *M. nipponense* muscle. Our study suggests there is a need for the monitoring of metabolic profiles in prawn muscle in order to identify the overall prawn health and environmental conditions.

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