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Effect of supplemental L-ascorbyl-2-polyphosphate (APP) in enriched live food on the immune response of *Penaeus vannamei* exposed to ammonia-N

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Abstract

The effects of supplemental vitamin C, in the form L-ascorbyl-2-polyphosphate (APP) in enriched live food dietary (*Artemia*) on reactive oxygen intermediates (ROIs) and free radical scavenging enzymes (such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione transferase) activities in muscle of *Penaeus vannamei* exposed to ambient ammonia-N were investigated. The results showed ROIs values of shrimps fed the starved and enriched *Artemia* increased with increased ammonia-N concentration. The ROIs value of shrimps fed the enriched *Artemia* exposed to increasing ammonia-N concentration were 37.4%, 26.4% and 31.1% lower ($P<0.05$) compared with those of shrimps fed the starved *Artemia* exposed to the same ammonia-N concentration. Total SOD, CAT, GPX, GST and GR activities of shrimps fed the enriched *Artemia* exposed to ammonia-N (2.568–3.852 mmol/l), were all higher ($P<0.05$) than that of shrimps fed the starved *Artemia* exposed to the same ammonia-N concentration. In addition, total SOD, CAT, GPX, GST and GR activities of shrimps of both dietary treatments decreased ($P<0.05$) with increased ammonia-N concentration. The results demonstrated that supplementation of ascorbic acid in enriched live food (*Artemia*) enhanced the anti-oxidant capacity of shrimp, increasing its defense system that may fight against environmental stress leading to reduced ammonia toxicity.

Keywords: *Penaeus vannamei*; Reactive oxygen intermediates; Antioxidant enzymes; Ammonia-N; Vitamin C

1. Introduction

Vitamin C is an important component of aquatic organisms. It is widely distributed in intracellular and extracellular fluids and it takes part in some physiology metabolisms. It functions as a general water-soluble redox reagent, a cofactor in collagen synthesis, a regulator of steroid synthesis, a growth activator in wound healing, a modulator of the hexose monophosphate shunt, and an inactivator of hepatic microsomal hydroxylases (Panush and Delafuente, 1985). A lack of L-gulonolactone oxidase, an enzyme required for biosynthesis of ascorbate from glucose as a precursor, leads to reliance upon exogenous sources of vitamin C (Panush and Delafuente, 1985). Dietary vitamin C is essential for penaeid shrimp and its deficiency would induce severe damage, such as the "black death" syndrome (Lightner et al., 1979). It also acts as a promising nutritional supplement, since it has been

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already demonstrated that this vitamin, besides functioning as a potent antioxidant (Block and Langseth, 1994), such as an immunostimulant in fish and crustacean (Merchie et al., 1997a; Lee and Shiau, 2002; Maggioni et al., 2004). A further improvement of the nutritional quality of *Artemia* via vitamin C enrichment has been demonstrated by Merchie et al. (1995a,b; 1997b).

Vitamin C is considered the most important antioxidant in extracellular fluids being capable of scavenging oxygen-derived free radicals. Thereby, it inhibits lipid peroxidation initiated by peroxyl radicals in aqueous media and regulates the extracellular redox state through its interaction with glutathione (Rinne et al., 2000). Many physical and chemical stresses can arouse free radical production.

Exposure to environmental stressors is an inescapable aspect of an aquatic organism's life. These stresses include temperature, pH, light, oxygen, salt, pollutant, and so on. Among all above, ammonia is very toxic to aquatic animals and cause damage. Ammonia, the end product of protein catabolism, accounts for more than half the nitrogenous waste released by decapod crustaceans. The toxicity of ammonia to crustaceans has been studied by several authors (Chen and Lin, 1991; Wajsbrot et al., 1990; Wang et al., 2003). Elevated concentration of environmental ammonia has been reported to affect growth and molting (Chen and Kou, 1992), oxygen consumption, ammonia excretion and Na^+/K^+ ATPase (Wang et al., 2003). Ammonia has also been reported to affect the immune response of *Litopenaeus stylirosyris* (Le Moullac and Haffner, 2000), *Macrobrachium rosenbergii* (Cheng and Chen, 2002) and *Macrobrachium nipponese* (Wang et al., 2005).

Most of the superoxide anions (O_2^-) produced by mitochondria are converted to H_2O_2 mitochondrial SOD activity. GPX catalyzes specially the reaction of reduced glutathione (GSH) to oxidized glutathione (GSSG) and the reduction of hydrogen peroxide. CAT catalyzes the decomposition of H_2O_2, if H_2O_2 was not scavenged in time, it could turn into hydroxyl radicals. The antioxidant enzymes also include glutathione reductase (GR) and glutathione *S*-transferase (GST). GR catalyzes GSH to GSSG, and GST catalyzes the conjugation of reduced glutathione (GSH) to nucleophilic xenobiotics or cellular components damaged by oxyradicals attack (Pang et al., 2000).

The aim of this study was to assess the effect of vitamin C on some *P. vannamei* immune response under ammonia stress, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione transferase (GST), and glutathione reductase (GR).

2. Materials and methods

Shrimps *Penaeus vannamei* were obtained from a commercial shrimp hatchery in Hebei Province, China, in June 2001, and were transferred and acclimated for 2 weeks. Shrimps of each dietary treatment, averaging 1.93 ± 0.202 cm, 0.1243 ± 0.0355 g, were kept in $30 \times 20 \times 15$ cm glass tanks (seventy shrimps/tank) with seawater at 30 g l^{-1}, pH 8.0 and controlled temperature (25 ± 2 °C). Two replicate tanks were assigned to each dietary treatment.

2.1. Feeding trials

Shrimps were fed *Artemia franciscana* nauplii enriched with L-ascorbyl-2-polyphosphate (APP); a control group shrimps were fed untreated *Artemia* nauplii. *Artemia franciscana* nauplii (origin: Great Slat Lake, UT, USA) were hatched before feeding and placed in 1 l plastic beaker which contained seawater and 480 mg APP (equal 120 mg ascorbic acid). The APP quantity was according to He and Lawrence (1993). Enrichment lasted for 40 min. After that, *Artemia* nauplii were counted and administered to the aquaria, approximately 6 individuals ml^{-1}. Remaining *Artemia* nauplii were removed after 2 h. Feeding was done three times a day at 9:00, 15:00 and 21:00 respectively. Shrimps were weighed at the beginning of the experiment and after 20 days.

2.2. Ammonia toxicity test

After 20 days, the starved 2-day intermolt shrimps determined by Chan et al. (1988) were used for a short-term toxicity test of ammonia-N. The shrimps were transferred to plastic tanks containing 5 l of a series test solutions (1.284 mmol/l, 2.568 mmol/l, 3.852 mmol/l) prepared by appropriate dilutions from a stock solution of NH_4Cl dissolved in filtered seawater (Chen and Lin, 1992). These concentrations are considered sublethal for *P. vannamei* juveniles. The 24 h-LC_{50} is 4.28 mmol/l under the same conditions (Racotta and Hernández-Herrera, 2000). Each test solution had three replicates, and was aerated through an air blower attached to a bubble stone. Ammonia-N was determined by the phenolhypochlorite method (Solorzano, 1969).

After 24 h, the reactive oxygen intermediates production in muscle of the shrimp was determined and modified by Muñoz et al. (2000). Before measuring,