Available online at www.sciencedirect.com
ScienceDirect
Aquaculture
Aquaculture 261 (2006) 1432–1439
www.elsevier.com/locate/aqua-online

Review Article

Effect of saponin on hematological and immunological parameters of the giant freshwater prawn, *Macrobrachium rosenbergii*

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Received 7 June 2006; received in revised form 31 August 2006; accepted 31 August 2006

Abstract

Giant freshwater prawns, *Macrobrachium rosenbergii* (17.9 ± 2.7 g), exposed to different concentrations of saponin at 0, 0.3, 0.6, 0.9 and 1.2 mg l^{-1} for 168 h were examined for osmolality, electrolyte levels, oxyhemocyanin, protein levels, acid-base balance status, total hemocyte count (THC), phenoloxidase activity, and respiratory bursts. Hemolymph oxyhemocyanin, protein, and pO_2 were inversely related to the saponin concentration. Hemolymph oxyhemocyanin, protein, pO_2, pCO_2, and pH of prawns exposed to 1.2 mg l^{-1} saponin were significantly lower than those of prawns exposed to 0.3 mg l^{-1} and control solutions. However, no significant difference was observed in osmolality or electrolyte levels of prawns exposed to different concentrations of saponin for 168 h. The THC of prawns following 168 h of exposure to 0.9 and 1.2 mg l^{-1} saponin increased, but the phenoloxidase activity decreased suggesting that the decrease in phenoloxidase activity under saponin stress was not a consequence of the increase in THC. We concluded that saponin at as low as 0.9 mg l^{-1} decreases the respiratory protein level and acid-base balance, and modulates the immune system of *M. rosenbergii*.

Keywords: *Macrobrachium rosenbergii*; Hemolymph; Oxyhemocyanin; Acid-base balance; Hemocyte count; Phenoloxidase activity; Respiratory burst

Contents

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0044-8486/$ - see front matter
doi:10.1016/j.aquaculture.2006.08.051

1. Introduction

The giant freshwater prawn, *Macrobrachium rosenbergii*, is commercially important in the world as a primary inland cultured species. Disease outbreaks caused by yeast infections in the cool season and bacteria in the hot season have resulted in declining production of farmed prawns in Taiwan (Hsu, 1993; Cheng and Chen, 1998; Chen et al., 2001).

Several studies demonstrated the disruption of normal osmotic and ionic balance after exposure to pollutants (Caldwell, 1974; Inman and Lockwood, 1977; Neufeld and Pritchard, 1979), and osmoregulatory capacity has been proposed as a potential indicator of the physiological condition and a stress indicator among crustaceans (Boitel and Truchot, 1989; Charmantier et al., 1989; Young-Lai et al., 1991; Lin et al., 1993; Bambang et al., 1995a,b). Hemolymph hemocyanin protein levels, and the acid-base balance of crustaceans are altered by changes in ambient salinity, temperature, dissolved oxygen (Truchot, 1983; Ferraris et al., 1986), ammonia-N (Chen and Cheng, 1993a), and saponin (Chen and Chen, 1996). Previous studies also indicated that both sublethal and lethal concentrations of trichlorfon affect the hemolymph acid-base balance, osmolality, and ion concentrations in *M. rosenbergii* kept in freshwater systems (Yeh et al., 2005; Chang et al., 2006).

In decapod crustaceans, hemocytes are involved in phagocytosis, which eliminates microbes or foreign particles (Bachère et al., 1995; Johansson, 1995). Hemocytes are associated with proteins like prophenoloxidase (proPO) which is involved in encapsulation, melanization, and cytotoxicity as a non-self recognition system (Johansson and Söderhäll, 1989). Phenoloxidase is the terminal enzyme in the proPO activation system and is activated by several microbial polysaccharides, including β-1,3-glucan from fungal cell walls (Smith et al., 1984). A high pH and low dissolved oxygen (DO) have been reported to cause reductions in hemocyte counts in *M. rosenbergii* (Cheng and Chen, 2000; Cheng et al., 2002b) and in blue shrimp *Litopenaeus stylirostris* (Le Moullac et al., 1998). Primary physicochemical changes like high temperature, high pH, and low dissolved oxygen, and environmental toxicants like ammonia, nitrite, and copper sulfate have been reported to decrease the phenoloxidase activity of *M. rosenbergii* (Cheng and Chen, 2000, 2002; Cheng and Wang, 2001, Cheng et al., 2002a,b).

Reactive oxygen species (ROS) like superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-) are produced during phagocytosis. This phenomenon, known as a respiratory burst, plays an important role in microbicidal activity (Song and Hsieh, 1994). The generation of O_2^- has been reported in hemocytes of tiger shrimp, *Penaeus monodon* (Song and Hsieh, 1994), *L. stylirostris* (Bachère et al., 1995), and white shrimp, *Litopenaeus vannamei* (Muñoz et al., 2000). Environmental toxicants like ammonia, nitrite, copper sulfate, and trichlorfon have been reported to increase the release of superoxide anions in *M. rosenbergii* (Cheng and Wang, 2001; Cheng and Chen, 2002; Cheng et al., 2002a; Chang et al., 2006).

Tea seed cake, the residue of *Camellia* sp. seeds after oil extraction contains 5.2%–7.2% saponin (Minsalan and Chiu, 1986). Tea seed cake is widely used as a piscicide. The application of tea seed cake is very effective in eradicating predatory fish in prawn ponds (Terazaki et al., 1980). An application rate of tea seed cake of 15 mg l^{-1} is considered suitable (Minsalan and Chiu, 1986). Since prawn farmers often apply excess amounts of tea seed cake in pond management, the concentration of saponin in the water and its effect on the physiological and immune systems of cultured prawn are of primary concern. The 24- and 96-h LC_{50} values (median lethal concentration) of saponin to Kuruma shrimp, *Marsupenaeus japonicus*, juveniles have been reported to be 27.08 and 18.14 mg l^{-1}, respectively (Chen et al., 1996). Saponin has been reported to decrease the hemocyanin level and affect the acid-base balance of *M. japonicus* (Chen and Chen, 1996). However, little is known about the effect of saponin on lethality, physiological responses, and immune responses of *M. rosenbergii*.

In the present study, we attempted to examine physiological and immune responses of *M. rosenbergii* to