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Acute salinity stress alters the haemolymph metabolic profile of *Penaeus monodon* and reduces immunocompetence to white spot syndrome virus infection

Annies Joseph, Rosamma Philip *

Department of Marine Biology, Microbiology and Biochemistry, Cochin University of Science and Technology, Cochin-16, India

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Abstract

Influence of acute salinity stress on the immunological and physiological response of *Penaeus monodon* to white spot syndrome virus (WSSV) infection was analysed. *P. monodon* maintained at 15‰ were subjected to acute salinity changes to 0‰ and 35‰ in 7 h and then challenged orally with WSSV. Immune variables viz., total haemocyte count, phenol oxidase activity (PO), nitroblue tetrazolium salt (NBT) reduction, alkaline phosphatase activity (ALP), acid phosphatase activity (ACP) and metabolic variables viz., total protein, total carbohydrates, total free amino acids (TFAA), total lipids, glucose and cholesterol were determined soon after salinity change and on post challenge days 2 (PCD2) and 5 (PCD5). Acute salinity change induced an increase in metabolic variables in shrimps at 35‰ except TFAA. Immune variables reduced significantly ($P<0.05$) in shrimps subjected to salinity stress with the exception of ALP and PO at 35‰ and the reduction was found to be more at 0‰. Better performance of metabolic and immune variables in general could be observed in shrimps maintained at 15‰ that showed significantly higher post challenge survival following infection compared to those under salinity stress. Stress was found to be higher in shrimps subjected to salinity change to lower level (0‰) than to higher level (35‰) as being evidenced by the better immune response and survival at 35‰. THC ($P<0.001$), ALP ($P<0.01$) and PO ($P<0.05$) that together explained a greater percentage of variability in survival rate, could be proposed as the most potential health indicators in shrimp haemolymph. It can be concluded from the study that acute salinity stress induces alterations in the haemolymph metabolic and immune variables of *P. monodon* affecting the immunocompetence and increasing susceptibility to WSSV, particularly at low salinity stress conditions.

Keywords: *Penaeus monodon*; White spot syndrome virus; Salinity; Haemolymph; Immune response

1. Introduction

White spot syndrome, first reported in Taiwan in 1992 (Chou et al., 1995), has emerged as the most serious threat to commercial shrimp farming. White spot syndrome virus (WSSV), a member of the genus Whispovirus within a new virus family Nimaviridae is a circular, double-stranded DNA virus (Vlak et al., 2005). Tiger shrimp, *Penaeus monodon*, the widely cultured shrimp species is highly susceptible to WSSV infection (Chen, 1995; Hameed et al., 2006). Susceptibility is often intensified by the highly stressful environment in culture systems.

Stress responses to environmental fluctuations are well reflected in the composition of haemolymph, the prime component involved in the defense mechanism of

* Corresponding author. Tel./fax: +91 484 2381120.
E-mail address: rose@cusat.ac.in (R. Philip).

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crustaceans. Haemocytes, along with proPhenol oxidase activity, respiratory burst activity and phagocytic activity have been used as indices of immune capability in penaeid shrimps (Le Moullac et al., 1998; Cheng et al., 2007). Extrinsic factors like temperature (Pascual et al., 2003; Wang and Chen, 2006b), salinity (Vargas-Albores et al., 1998), pH (Cheng and Chen, 2000) dissolved oxygen (Jiang et al., 2005) etc. are reported to affect immune responses in several species of decapod crustaceans. Biochemical variables in haemolymph have also been identified as indicators of stress as stress leads to the onset of a cascade of molecular and biochemical responses. Haemolymph metabolic variables viz., proteins, glucose, cholesterol, triacylglycerol etc. were found to vary in response to captivity stress (Sanchez et al., 2001), temperature alterations (Pascual et al., 2003), depleted dissolved oxygen (Hall and van Ham, 1998), high ambient ammonia (Racotta and Hernandez-Herrera, 2000) etc. Stress therefore disrupts the immune ability and metabolic performance of shrimps, increasing the susceptibility to microbial infecions. However, there are very few scientific data supporting the link between environmental stress and increased susceptibility to diseases in shrimps.

P. monodon with an iso-osmotic point of 750 mOsm kg^{-1} (equivalent to 25‰) is very often cultured at a salinity range of 10‰–20‰, as they are believed to exhibit better growth in brackish water than in pure seawater under culture conditions (Fang et al., 1992). Being a euryhaline form having wide salinity tolerance ranging from 1‰ to 57‰ (Chen, 1990), the fluctuations are usually neglected in culture ponds. The salinity of culture ponds may decrease suddenly to as low as 0‰ after a heavy rainfall. There are reports of WSSV outbreaks with the onset of monsoon in Malaysia when intense rainfall decreased the salinity of aquaculture areas (Oseko, 2006). It is possible that acute salinity changes over a particular range weaken the immune system of shrimp and make them highly vulnerable to pathogens. Drastic salinity changes may also affect the feed intake, metabolism, and higher energy utilization for osmoregulation resulting in poor growth. However, there are very few works on the effects of environmental stress on infection, particularly WSSV.

Present study on *P. monodon* was therefore aimed at determining the: (i) effect of acute salinity change on the metabolic and immune variables of haemolymph (ii) effect of acute salinity stress on the susceptibility to WSSV infection. (iii) effect of WSSV infection on the haemolymph metabolic variables and immune response of shrimps maintained at optimal salinity and those subjected to acute salinity stress.

2. Materials and methods

2.1. Experimental animals and rearing conditions

Adult *P. monodon* obtained from a commercial farm in Panangad, Kochi were used as experimental shrimps in the present study. They were transported to the laboratory within 1 h of capture. Average wet weight of the shrimp was 19.85± 2.01 g (Mean±S.D.). Shrimps were reared in rectangular concrete tanks containing 15‰ sea water and allowed to acclimate for a week. Continuous aeration was provided and shrimps were fed on a commercial shrimp diet (Higashimaru, Kochi). Water quality parameters viz., salinity, temperature, dissolved oxygen, NH_3-N, NO_2-N and NO_3-N were monitored daily following standard procedures (APHA, 1995) and maintained at optimal levels as per Table 1. Unused feed and faecal matter were siphoned out daily and 25% water exchanged every second day. A biological filter was set up to maintain the appropriate levels of water quality parameters. After acclimation for a period of 7 days, the biochemical and immunological profile was obtained from a group of shrimps ($n=6$) as the baseline (BL) data.

2.2. Experimental design

Shrimps were distributed in the experimental tanks containing 500 L of seawater ($n=30$/tank). Shrimps in the intermoult stage (Robertson et al., 1987) only were used. There were 4 treatments (Group-I, Group-II, Group-III and Group-IV) and the experiment was conducted in triplicate i.e., 3 tanks per treatment. Salinity of all the tanks was adjusted to 15‰ prior to the experiment.

2.3. Salinity stress

After 2 days, the shrimps of Group-II and Group-IV were subjected to sudden salinity changes. Shrimps were starved for 12 h prior to salinity change. Salinity of Group-II was lowered from 15‰ to 0‰ by diluting with fresh water. Whereas, the salinity of Group-IV was raised from 15‰ to 35‰ by adding sea water. The desired salinity was adjusted

Table 1
Rearing conditions and water quality

Stocking density	30 shrimps/tank
Tank capacity	500 L
Feeding level	10–15% body weight
Feeding frequency	twice daily
Water temperature	24–27 °C
pH	7.5–8.0
Salinity	15‰
NH_3-N	0.01–0.02 mgl^{-1}
NO_3-N	below detectable level
NO_2-N	0.00–0.01 mgl^{-1}
Dissolved oxygen	6–7 mgl^{-1}