Available online at www.sciencedirect.com
SCIENCE DIRECT
Comparative Biochemistry and Physiology, Part A 142 (2005) 10 – 18
CBP
www.elsevier.com/locate/cbpa

Dietary high protein and vitamin C mitigate stress due to chelate claw ablation in *Macrobrachium rosenbergii* males

S.M. Manush, A.K. Pal *, T. Das, S.C. Mukherjee

Fish Physiology, Pharmacology and Therapeutic Cell, Central Institute of Fisheries Education, Fisheries University Road, Versova, Mumbai-400 061, India

Received 7 April 2005; received in revised form 9 July 2005; accepted 12 July 2005
Available online 16 August 2005

Abstract

Stress due to claw ablation was tested in *Macrobrachium rosenbergii* males. Dietary high protein and vitamin C were supplemented for amelioration of stress. We used four different treatments: fed with 25% protein and a normal dose (0.12%) of vitamin C (T_1); 35% protein and a normal dose (0.12%) of vitamin C (T_2); 25% protein and a high dose (0.24%) of vitamin C (T_3); and high protein 35% and a high dose (0.24%) of vitamin C (T_4) for 30 days. All test prawns (T_1 to T_4) were subjected to ablation of their second chelate legs after the 15th day of the feeding trial. A control treatment was maintained without claw ablation and fed with 25% protein. Haemolymph glucose, hepatopancreatic glycogen, muscle ascorbate and enzyme activities (glucose 6 phosphatase (G6Pase), fructose-1,6-bisphosphatase (FBPase), lactate dehydrogenase (LDH), Alanine aminotransferase (ALT) in hepatopancreas) were tested at different recovery periods (0, 6, 24 h, 7 and 14 days). Results indicate a high glucose level immediately after claw ablation and a concomitant increase in gluconeogenic enzymes (G6Pase and FBPase). However, glycogen reserves were regained in the treatments due to claw ablation stress after 24 h. LDH and ALT activity decreased in the hepatopancreas of *M. rosenbergii* up to 24 h after claw ablation. Overall results indicate that claw ablation is stressful to *M. rosenbergii* and high protein and vitamin C diet may mitigate stress due to claw ablation.

Keywords: Stress; Claw ablation; Protein; Ascorbic acid; Mitigation; Glucose; Glycogen; Metabolic enzymes; Freshwater prawn; *Macrobrachium rosenbergii*

1. Introduction

Freshwater prawn farming has become a significant and valuable sector of global aquaculture, contributing 0.3 million MT of all *Macrobrachium* species raised through aquaculture and is being increased with an annual expansion rate of 48% between 1999 and 2001 (New, 2003). The world production of *Macrobrachium rosenbergii* increased from 26,588 MT in 1991 to 118, 501 MT in 2000 (FAO, 2002). In India, the total annual production of freshwater prawns (*M. rosenbergii* and *M. malcomsonii*) increased from 7140 to 30,450 MT (4.3 fold increase) and a corresponding increase in the area of prawn culture from 12,022 to 34, 630 ha (2.9 fold increase) from 1999–2000 to 2002–2003, respectively (MPEDA, 2003), which clearly indicates the importance of this species in freshwater aquaculture. However, differential growth is one of the main limitations of *M. rosenbergii* monoculture in pond conditions.

Male prawns exhibit a complex bimodal population structure, with small males (SM) or runts, orange-clawed (OC) males and blue clawed (BC) males or bulls, which differ in size, morphology, physiology and behaviour (Ra'anan and Sagi, 1985; Kuris et al., 1987; Sagi and Ra'anan, 1988). *M. rosenbergii* males have been described as very aggressive (John, 1957; Ra'anan and Sagi, 1985). BC males are highly aggressive, dominant and territorial while OC males are considered aggressive, sub-dominant, and non-territorial in nature. Autotomy as a result of damage or injury is well developed in *M. rosenbergii*. To reduce

* Corresponding author. Tel.: +91 22 6361446; fax: +91 22 26361573.
E-mail address: akpal_53@rediffmail.com (A.K. Pal).

1095-6433/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpb.2005.07.004

cannibalism and improve uniform growth among male prawns, various management practices like batch culture (stocking once and selective harvesting at different intervals followed by drain harvesting at the end of culture period) are being carried out in various parts of the world (New, 2003). Another method of partial or complete claw ablation or claw immobilization has revealed marked increase in survival and uniformity of growth in communally raised American lobster, *Homarus americanus* (Aiken and Young Lai, 1979, 1981; Kendall et al., 1982) and *M. rosenbergii* (Karplus et al., 1989). However, no convincing reports on stress associated with claw ablation have been demonstrated in *M. rosenbergii.*

Growing intensification of aquaculture over the last decade is a striking example where stress and its response assume immediate economic importance (Pickering, 1981). Dietary high protein has ameliorating effects against different stressors. It is reported that supplementation of high protein (50%) and vitamin C (0.2%) reduces bioaccumulation and stress responses due to endosulfan toxicity in *Channa punctatus* (Sarma, 2004). Vitamin C is considered to play an important role in animal health as antioxidant (Chew, 1995). Vitamin C increases resistance of shrimp to bacterial infections (Kanazawa, 1996). Shrimp increased resistance to *Vibrio harveyii* infection after 18 days, while shrimp receiving high ascorbate levels exhibited no mortality (Kontara et al., 1997). Vitamin C enhanced tolerance to hypoxic stress (Ishibashi et al., 1992) and increased immunoresistance (Li and Lovell, 1985; Navarre and Halver, 1989; Hardie et al., 1991; Obach and Laurencin, 1992). Against this background, the present investigation was undertaken to assess the stress due to claw ablation in *M. rosenbergii* males. Dietary high protein and vitamin C were supplemented during recovery of claw ablated *M. rosenbergii* to improve the regeneration of chelate claws.

The gluconeogenic pathway is active in fishes since they prefer to utilize the protein and lipids rather than carbohydrate for energy requirements (Demeal, 1978). However, in fish under stress, glucose is mainly derived by gluconeogenesis (Vijayan et al., 1993). It is not clear whether crustacean hyperglycemic hormone induces glycogen breakdown for meeting energy requirements under stressed condition. Therefore, we tested two key gluconeogenic enzymes; glucose-6 phosphatase (G6Pase) and fructose-1,6-bisphosphatase (FBPase) during recovery phase of claw ablation. Another enzyme, lactate dehydrogenase (LDH) is the terminal enzyme of glycolysis, responsible for reversible conversion of pyruvate to lactate and is present in most tissues. An increase of LDH activity is expected under stressed condition. Enzymes of protein metabolism (transaminase enzymes) are active in fishes and shellfishes as the energy is derived mainly from the protein source. Alanine aminotransferase (ALT), is responsible for transamination of alanine, a preferred substrate for gluconeogenesis (Suarez and Mommsen, 1987; Moon and Foster, 1995).

2. Materials and methods

2.1. Experimental animals

Blue claw males of *M. rosenbergii* (inter-molt stage of mean mass = 29 ± 3.1 g (±S.E.) were acclimatized to laboratory conditions for 30 days prior to the experiment, during which they were fed with control feeds (25% protein).

2.2. Experimental set up

A feeding trial was carried out in fibre-glass reinforced plastic pools (500 L). 200 Prawns were distributed in five different groups (control, T_1, T_2, T_3, T_4) with four tanks per treatment, each stocked with ten prawns/tank. They were fed with 25% protein (T_1) and a normal dose of vitamin C (0.12%), 35% protein and a normal dose (0.12%) of vitamin C (T_2), 25% protein and a high dose (0.24%) of vitamin C (T_3) and high protein 35% and a high dose (0.24%) of vitamin C (T_4) for 30 days. All prawns of test tanks (T_1 to T_4) were subjected to ablation of their second chelate legs after 15th day of feeding trial, aseptically using an electorcauteriser. Eight prawns were sampled from each treatment at different intervals (0 h, 6 h, 24 h, 7 days and 14 days) for collection of hemolymph and vital organs. A control treatment was maintained containing prawns without claw ablation and fed with 25% protein for 30 days. To prevent handling stress confound stress due to claw ablation, control prawns were also netted and handled like that of treatment prawns. However, in this experiment, no negative controls were maintained to assess stress due to handling alone. The experiment was carried out at optimum temperature conditions (30 °C) for *M. rosenbergii* (Sebastian, 1996). Continuous aeration was provided in all the experimental units from a centralized aeration unit to maintain the optimum level of dissolved oxygen as reported (Boyd and Zimmermann, 2000). Adequate shelters (2 in. PVC pipes) were provided to prevent cannibalism during the feeding trial.

2.3. Experimental diet

All ingredients were dried in a hot air oven at 80 °C for 8 h, powdered and sieved through 60 μm mesh size nylon netting. Proximate analysis of all the ground ingredients used for basal diet was carried out to formulate experimental diets. Five different diets were formulated (Table 1). Rovimix Stay-C 35 (Roche) was used as the vitamin C source. All the ingredients were mixed thoroughly with required amount of water (20%) to make dough, which was transferred to an aluminum container and placed in a pressure cooker for cooking for about 25 min. After cooling, vitamins and minerals were added to the dough and mixed thoroughly. The dough was pressed through a hand pelletizer (0.2 mm). Pellets were initially sun dried and later oven dried at 60 °C