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Comparative Biochemistry and Physiology, Part A 142 (2005) 10 – 18

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

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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

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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

Table 1
Composition of different experimental diets

Ingredients (%)	Different diets				
	Control	T_1	T_2	T_3	T_3
Fish meal	11.9	11.9	23.8	11.9	23.8
Soybean meal	11.9	11.9	23.8	11.9	23.8
Rice bran	38.0	38.0	26.1	38.0	26.1
Wheat flour	38.0	38.0	26.1	38.0	26.1
Fish oil	6.0	6.0	6.0	6.0	6.0
Vitamin–Mineral mix[1]	1.0	1.0	1.0	1.0	1.0
Vitamin B complex[2]	0.05	0.05	0.05	0.05	0.05
Vitamin C	0.12	0.12	0.12	0.24	0.24

[1]Composition of vitamin–mineral mix (Agrimin) (quantity kg^{-1}). Vitamin A —6,25,000 IU; Vitamin D_3 —62,500 IU; Vitamin E —250 mg; Nicotinamide —1 g; Cu —312 mg; Co —45 mg; Mg —6 g; Fe —1.5 g; Zn —2.13 g; I —156 mg; Se —10 mg; Mn —1.2 g; Ca —247.34 g; P —114.68 g; S —12.2 g; Na —5.8 mg; K —48.05 mg.
[2]Composition of vitamin B complex (quantity g^{-1}). Thiamine mononitrate —20 mg; Riboflavin —20 mg; Pyridoxine hydrochloride —6 mg; Vitamin B_{12} —30 mcg; Niaciamide —200 mg; Ca pantothenate —100 mg; Folic acid —3 mg; Biotin —200 mcg.

overnight. Feeding was done up to satiation and adjusted on daily observation of feed intake of post-larvae.

2.4. Proximate analysis

Dry weight of the feed and of the carcass were estimated after drying at 105 °C for 12 h and ashing (6 h at 600 °C). Crude fat (CF) content of the samples was assessed using Soxtec system (Model HT2, 1045 extraction unit Foss Tecator, Sweden) using diethyl ether as a solvent. Nitrogen in feed and carcass were estimated by a micro Kjeldahl method (Foss Tecator 2200 Kjeltec) and crude protein (CP) was calculated as $N \times 6.25$ (Chang, 1994). Total carbohydrate was calculated by difference, i.e., total carbohydrate (%) = 100 − (CP% + CF% + ash%). The digestible energy (DE) of the feed and of the carcass was calculated following Halver (1976).

2.5. Metabolic studies

2.5.1. Sample preparation

Prawns were sampled 0 h, 6 h, 24 h, 7 days and 14 days from different treatments (T_1, T_2, T_3, and T_4) and control after being anaesthetized with CIFECALM (200 μL L^{-1}). CIFECALM is a herbal anaesthetic formulation containing natural alcoholic extracts of *Eugenia caryophyllata,* and *Mentha arvensis* (developed by Central Institute of Fisheries Education, Mumbai). Haemolymph (four/treatment) for glucose estimation was collected from the coelomic cavity below cephalothoracic region on the dorsal side, using a hypodermic syringe. Pre-weighed hepatopancreas (0.3 g) were dissected out from remaining prawns sampled (four/treatment) and was dissolved in KOH (30%) and used for glycogen estimation. Samples of hepatopancreas and muscle (0.5 g) were homogenized in chilled sucrose solution (0.25 M) in a mechanical tissue homogenizer and centrifuged (3000 g at 4 °C for 10 min). Supernatant was collected and frozen (−20 °C) for enzymatic studies.

2.5.2. Glucose, glycogen and ascorbic acid

Glucose was estimated at 540 nm using arsenomolybdate reagent (Nelson-Somogyi, 1944–1945) Hepatopancreatic glycogen was estimated colorimetrically at 590 nm by treating with anthrone reagent (Hassid and Abraham, 1957). Ascorbic acid was estimated from muscle at 540 nm using 2,4-dinitro-phenylhydrazine (Roe and Keuther, 1943).

2.5.3. Enzyme assays

Glucose-6 phosphatase (G6Pase) (D-Glucose 6-phosphate phosphohydrolase; E.C. 3.1.3.9) was estimated using malate buffer (pH 6.5) and 0.1 M glucose 6-phosphate solution. Fructose-1, 6-bisphosphatase (FBPase) (D-FBP-1-phosphohydrolase; E.C. 3.1.3.11) was estimated using 50 mM borate buffer (pH 9.5), fructose-1,6-bisphosphate (pH 7–7.3) and 0.5 M $MgSO_4$. Phosphate liberated by G6Pase and FBPase was estimated at 660 nm (Fiske and Subbarow, 1925). Lactate dehydrogenase (LDH) (L-Lactate NAD+oxidoreductase; E.C.1.1.1.27) was assayed in 100 mM phosphate buffer (pH 7.5), 0.1 mM NADH. The reaction was initiated by adding 0.2 M Na-pyruvate and monitored at 340 nm. Alanine amino transferase (ALT) (L-Alanine-2-oxaloglutarate aminotransferase; E.C.2.6.1.2) was assayed with 200 mM M D,L-alanine and 2 mM α-ketoglutarate in 40 mM phosphate buffer (pH 7.4) and estimated at 540 nm (Wooton, 1964). Total protein content was analyzed from the supernatant (Lowry et al., 1951) for calculating enzyme activities.

2.5.4. Statistical analyses

Recovery from stress due to claw ablation at different intervals (0 h, 6 h, 24 h, 7 days and 14 days) and proximate composition of feed samples were investigated by using one-way ANOVA. The effect of dietary supplementation (high protein and vitamin C) on regeneration of claws and proximate composition of carcass tissue was tested by using two-way ANOVA. Post hoc test in all the cases were carried out using Duncan's multiple comparison procedures, if they were significantly different. All the statistical analyses were performed with SPSS 11.0 for Windows.

3. Results

3.1. Proximate composition

Data on proximate composition of feeds of *M. rosenbergii* fed during claw ablation and mitigation using dietary high protein and vitamin C and carcass tissue at different sampling intervals are presented in Tables 2 and 3 (a and b) respectively. Crude protein of experimental diets demonstrated significantly ($p < 0.05$) higher values in T_2 and T_4 (high protein diets). Carcass tissue demonstrated a

Table 2
Different superscripts (a, b, c) in the same row indicate significant difference ($p<0.05$) (overall mean values) between different treatments (control, T_1, T_2, T_3 and T_4)

	Treatment feeds				
	Control	T_1	T_2	T_3	T_4
Moisture	9.69±1.87	9.84±1.01	6.93±0.27	8.36±0.53	9.42±0.52
Organic matter	95.23±3.04^{a}	85.99±0.42^{b}	86.08±0.16^{b}	85.23±0.19^{b}	85.00±0.57^{b}
Crude protein	25.03±0.17^{a}	24.01±0.05^{b}	34.87±0.17^{c}	24.61±0.24^{ab}	33.82±0.35^{d}
Ether extract	12.53±0.33^{a}	12.1±0.14^{ab}	11.48±0.1^{b}	12.28±0.11^{a}	11.55±0.23^{b}
Ash	13.45±0.34^{a}	14.00±0.42^{ab}	13.92±0.16^{ab}	14.77±0.19^{b}	15.00±0.57^{b}
Total carbohydrate	48.99±0.37^{ab}	49.88±0.32^{a}	39.73±0.31^{c}	48.35±0.49^{b}	39.63±0.63^{c}
Digestible energy	462.67±1.66^{a}	460.5±0.68^{ab}	457.37±0.48^{b}	461.38±0.54^{a}	457.76±1.17^{b}

Proximate composition is expressed as % dry matter basis. Total carbohydrate (%)=100−(CP%+CF%+ash%). Digestible energy (Kcal/100 g)=Protein (%)×4+Lipid (%)×9+Carbohydrate (%)×4 (Halver, 1976). Values are expressed as mean±SE ($n=4$).

concomitant increase in crude protein levels with respect to dietary protein supplementation (Table 3a).

3.2. Glucose, glycogen and ascorbate

Data on glucose, glycogen and ascorbic acid of *M. rosenbergii* during claw ablation and mitigation using dietary high protein and vitamin C are depicted in Table 4. Higher glucose levels were recorded in all treatment groups (T_1 to T_4) immediately after claw ablation (0 h). Further, a decreasing trend of glucose level was depicted in all treatment groups (T_1 to T_4) until 24 h followed by an increased glucose level ($p<0.05$). In the control group, the highest glucose level was observed on the 7th day after claw ablation. An increase in glucose level was depicted in treatment groups (T_1 to T_4) over the control group irrespective of the recovery period. A decrease in glycogen level was demonstrated until 6 h in all the treatment groups (T_1 to T_4) and was significantly different ($p<0.05$). However, change in glycogen levels did not follow a definite decreasing trend as compared to the increasing glucose levels. A recovery of ascorbic acid levels was recorded in all treatment groups (T_1 to T_4) after 6 h. Further, a decreasing trend of ascorbic acid levels was observed until 7 days and was significantly different ($p<0.05$). However, tissue deposition of ascorbic acid was concomitant with dietary ascorbic acid levels (T_3 and T_4>control) irrespective of the recovery period.

Table 3a
Proximate composition of carcass of *M. rosenbergii* after claw ablation and mitigation studies using high protein and vitamin C diet

	Treatment feeds					
	Recovery period	Control	T_1	T_2	T_3	T_4
Moisture	0 h	75.22±0.72	79.93±0.26^{a}	75.58±0.33^{ab}	77.49±0.15^{a}	77.56±0.06^{a}
	6 h	75.72±0.2	75.45±0.39^{b}	76.1±0.3^{ab}	76.46±0.62^{a}	74.51±0.91^{b}
	24 h	74.71±1.1	75.45±1.27^{b}	76.66±0.72^{a}	58.34±0.87^{b}	74.13±1.03^{b}
	7 days	75.51±1.03	76.85±0.19^{b}	76.89±1.12^{b}	76.13±0.15^{a}	77.22±0.38^{a}
	14 days	75.97±0.15	79.98±1.02^{b}	77.23±0.22^{b}	73.63±1.07^{c}	78.76±0.65^{a}
	Over all mean	75.43±0.32^{A}	76.93±0.48^{B}	76.09±0.33^{AB}	72.41±1.66^{C}	76.44±0.49^{B}
Organic matter	0 h	94.51±0.38^{ac}	93.37±0.55^{a}	94.33±0.06^{a}	94.91±0.4^{a}	93.87±0.05^{ab}
	6 h	93.45±0.35^{ab}	95.02±0.09^{b}	94.77±0.39^{ab}	94.36±0.13^{a}	95.89±0.08^{c}
	24 h	93.12±0.43^{b}	95.97±0.1^{c}	94.07±0.23^{a}	94.97±0.41^{a}	94.29±0.26^{d}
	7 days	93.64±0.26^{ab}	93.09±0.04^{a}	95.24±0.39^{b}	94.96±0.24^{a}	93.97±0.09^{ab}
	14 days	95.43±0.44^{c}	94.97±0.37^{b}	94.22±0.09^{a}	96.3±0.08^{b}	93.58±0.11^{a}
	Over all mean	94.03±0.24^{A}	94.48±0.28^{B}	94.53±0.15^{B}	95.1±0.19^{C}	94.32±0.19^{AB}
Crude protein	0 h	79.37±0.26^{a}	75.47±0.25^{a}	75.58±0.26^{a}	78.09±0.41^{a}	77.96±0.26^{ab}
	6 h	78.92±0.26^{a}	44.54±1.84^{b}	74.78±0.39^{ab}	75.4±0.34^{b}	78.66±0.27^{a}
	24 h	78.17±0.82^{a}	78.98±0.26^{c}	74.28±0.45^{b}	75.06±0.35^{bc}	75.19±0.17^{c}
	7 days	66.82±0.83^{b}	72.54±0.25^{d}	77.11±0.26^{c}	74.04±0.63^{c}	77.67±0.24^{b}
	14 days	75.95±0.31^{c}	77.63±0.27^{ac}	79.15±0.14^{d}	77.2±0.14^{a}	80.57±0.42^{d}
	Over all mean	75.84±1.09^{A}	69.83±2.96^{B}	76.18±0.42^{A}	75.96±0.38^{A}	78.01±0.41^{C}
Ether extract	0 h	5.89±0.13^{a}	4.6±0.45^{a}	3.06±0.06^{a}	2.88±0.33^{a}	6.21±0.14^{a}
	6 h	4.25±0.06^{b}	2.39±0.13^{b}	5.19±0.3^{b}	4.54±0.14^{b}	6.45±0.21^{a}
	24 h	6.07±0.13^{a}	1.77±0.12^{bc}	4.59±0.14^{c}	1.26±0.08^{c}	2.48±0.19^{b}
	7 days	3.2±0.2^{b}	5.06±0.43^{a}	1.32±0.09^{d}	2.02±0.18^{d}	2.49±0.19^{b}
	14 days	3.56±0.84^{b}	0.93±0.07^{c}	1.35±0.06^{d}	1.71±0.13^{cd}	1.18±0.11^{c}
	Over all mean	4.59±0.31^{A}	2.95±0.39^{B}	3.1±0.37^{B}	2.48±0.28^{C}	3.76±0.49^{D}

Different superscripts (a, b, c, d) in the same column indicate significant difference amongst different recovery periods (Duncan's multiple range test, $\alpha=0.05$). Different superscripts (A, B, C, D) in the same row indicate significant difference ($p<0.05$) (overall mean values) between different treatments (control, T_1, T_2, T_3 and T_4). Values are expressed as mean ± SE ($n=4$). Values are expressed as mean ± SE ($n=4$). Proximate composition is expressed as % dry matter basis.

Table 3b
Proximate composition of carcass of *M. rosenbergii* after claw ablation and mitigation using high protein and vitamin C diet

	Treatment feeds					
	Recovery period	Control	T_1	T_2	T_3	T_4
Ash	0 h	5.49 ± 0.38^{ab}	6.64 ± 0.55^{a}	5.67 ± 0.06^{a}	5.09 ± 0.4^{a}	6.13 ± 0.05^{ab}
	6 h	6.55 ± 0.35^{bc}	4.98 ± 0.09^{b}	5.24 ± 0.39^{ab}	5.64 ± 0.13^{a}	4.1 ± 0.08^{c}
	24 h	6.89 ± 0.43^{c}	4.03 ± 0.09^{c}	5.93 ± 0.23^{a}	5.03 ± 0.41^{a}	5.71 ± 0.26^{a}
	7 days	6.37 ± 0.26^{bc}	6.91 ± 0.04^{a}	4.76 ± 0.39^{b}	5.04 ± 0.24^{a}	6.03 ± 0.09^{ab}
	14 days	4.57 ± 0.44^{a}	5.03 ± 0.37^{b}	5.78 ± 0.09^{a}	3.69 ± 0.08^{b}	6.43 ± 0.11^{b}
	Over all mean	5.97 ± 0.24^{A}	5.52 ± 0.28^{B}	5.47 ± 0.15^{B}	4.89 ± 0.19^{C}	5.68 ± 0.19^{AB}
Total carbohydrate	0 h	9.25 ± 0.22^{a}	13.29 ± 0.87^{a}	15.69 ± 0.17^{a}	13.94 ± 0.67^{a}	9.71 ± 0.39^{a}
	6 h	10.28 ± 0.52^{a}	48.09 ± 1.72^{c}	14.79 ± 0.47^{a}	14.43 ± 0.46^{a}	10.78 ± 0.41^{ab}
	24 h	8.88 ± 0.67^{a}	15.23 ± 0.13^{ab}	15.2 ± 0.27^{a}	18.66 ± 0.56^{b}	16.62 ± 0.33^{c}
	7 days	23.61 ± 0.44^{b}	15.49 ± 0.41^{ab}	16.81 ± 0.37^{b}	18.9 ± 0.55^{b}	13.81 ± 0.31^{d}
	14 days	15.93 ± 0.51^{c}	16.41 ± 0.51^{b}	13.73 ± 0.13^{c}	17.39 ± 0.31^{b}	11.83 ± 0.43^{b}
	Over all mean	13.59 ± 1.3^{A}	21.7 ± 0.06^{B}	15.25 ± 0.26^{C}	16.66 ± 0.52^{D}	12.55 ± 0.58^{E}
Energy	0 h	429.48 ± 0.62^{a}	423.00 ± 2.26^{a}	415.32 ± 0.28^{a}	414.42 ± 1.65^{a}	431.04 ± 0.69^{a}
	6 h	421.24 ± 0.29^{b}	411.93 ± 0.66^{b}	425.94 ± 1.52^{b}	422.68 ± 0.68^{b}	432.27 ± 1.06^{a}
	24 h	430.36 ± 0.64^{a}	408.82 ± 0.59^{bc}	422.97 ± 0.71^{c}	406.29 ± 0.39^{c}	412.37 ± 0.95^{b}
	7 days	416.02 ± 1.01^{b}	425.31 ± 2.12^{a}	406.61 ± 0.44^{d}	410.08 ± 0.88^{d}	412.46 ± 0.94^{b}
	14 days	417.79 ± 4.21^{b}	404.67 ± 0.37^{c}	406.73 ± 0.3^{d}	408.52 ± 0.65^{cd}	405.91 ± 0.54^{c}
	Over all mean	422.98 ± 1.57^{A}	414.75 ± 1.94^{B}	415.51 ± 1.86^{B}	412.39 ± 1.38^{C}	418.81 ± 2.5^{D}

Different superscripts (a, b, c, d) in the same column indicate significant difference amongst different recovery periods (Duncan's multiple range test, $\alpha=0.05$). Different superscripts (A, B, C, D) in the same row indicate significant difference ($p<0.05$) (overall mean values) between different treatments (control, T_1, T_2, T_3 and T_4). Values are expressed as mean ± SE ($n=4$). Proximate composition is expressed as % dry matter basis. Total carbohydrate (%) = 100 − (CP% + CF% + ash%). Digestible energy (kcal/100 g) = Protein (%) × 4 + Lipid (%) × 9 + Carbohydrate (%) × 4 (Halver, 1976).

3.3. Enzyme activities

Data on G6Pase and FBPase activity in hepatopancreas of *M. rosenbergii* during claw ablation are represented in Table 5. An increase in G6Pase (T_4) and FBPase activity was evident in the hepatopancreas up to 6 h after claw ablation in treatment groups (T_1), respectively. A similar increase in gluconeogenic enzymes was evident in high protein treatment groups (T_2 and T_4) including the controls after 7 days and was significantly different ($p<0.05$). An increase in G6Pase levels was evident in all treatment groups (T_1, T_2 and T_3) over control irrespective of the recovery period.

Data on LDH and ALT activity in hepatopancreas of *M. rosenbergii* during recovery phase after claw ablation are represented in Table 6. A decrease in LDH activity was

Table 4
Glucose, glycogen and ascorbic acid of *M. rosenbergii* in response to claw ablation and recovery using high protein and vitamin C diet

		Treatments					
		Recovery period	Control	T_1	T_2	T_3	T_4
Haemolymph	Glucose	0 h	44.62 ± 3.99^{ab}	99.53 ± 1.81^{a}	118.22 ± 5.94^{a}	122.43 ± 8.03^{a}	104.20 ± 6.08^{a}
		6 h	56.95 ± 7.53^{b}	71.65 ± 3.37^{b}	55.2 ± 3.89^{b}	65.66 ± 2.73^{bc}	53.5 ± 3.76^{b}
		24 h	79.44 ± 2.83^{c}	50.46 ± 5.27^{c}	50.0 ± 1.73^{b}	48.83 ± 9.6^{bd}	50.0 ± 2.43^{b}
		7 days	90.65 ± 1.48^{c}	124.07 ± 11.93^{d}	75.94 ± 10.7^{c}	78.27 ± 5.75^{c}	81.08 ± 3.48^{c}
		14 days	40.65 ± 1.73^{a}	80.6 ± 4.7^{ab}	61.92 ± 4.54^{bc}	34.11 ± 2.54^{d}	35.34 ± 3.48^{d}
		Over all mean	62.46 ± 4.77^{A}	85.26 ± 6.28^{D}	72.25 ± 6.14^{C}	69.85 ± 7.38^{BC}	64.82 ± 5.87^{AB}
Hepatopancreas	Glycogen	0 h	1.48 ± 0.19^{a}	2.29 ± 0.21^{a}	2.55 ± 0.16^{a}	2.7 ± 0.13^{a}	1.74 ± 0.04^{a}
		6 h	2.33 ± 0.08^{b}	1.01 ± 0.04^{b}	0.67 ± 0.02^{b}	0.97 ± 0.03^{b}	0.96 ± 0.02^{b}
		24 h	1.47 ± 0.04^{a}	0.96 ± 0.06^{b}	1.53 ± 0.09^{c}	0.54 ± 0.02^{c}	0.58 ± 0.05^{c}
		7 days	0.75 ± 0.04^{c}	0.77 ± 0.04^{b}	1.32 ± 0.1^{c}	1.08 ± 0.06^{b}	0.58 ± 0.04^{c}
		14 days	1.8 ± 0.06^{d}	0.42 ± 0.07^{c}	1.3 ± 0.07^{c}	1.71 ± 0.03^{c}	1.47 ± 0.09^{d}
		Over all mean	1.57 ± 0.12^{A}	1.09 ± 0.15^{C}	1.47 ± 0.15^{AB}	1.4 ± 0.17^{B}	1.06 ± 0.49^{C}
Muscle	Ascorbic acid	0 h	54.32 ± 11.51^{ab}	12.37 ± 1.01^{a}	15.6 ± 0.9^{a}	40.57 ± 5.63^{ab}	17.98 ± 1.66^{a}
		6 h	71.23 ± 16.01^{b}	42.33 ± 3.36^{b}	35.35 ± 2.4^{b}	44.77 ± 2.9^{a}	104.73 ± 3.3^{b}
		24 h	36.86 ± 2.36^{ac}	17.85 ± 2.26^{c}	22.79 ± 1.55^{c}	32.49 ± 2.58^{b}	55.66 ± 2.84^{c}
		7 days	21.27 ± 2.8^{c}	12.43 ± 1.78^{a}	22.37 ± 1.37^{c}	20.56 ± 2.02^{c}	42.97 ± 2.18^{d}
		14 days	40.39 ± 2.44^{ac}	54.3 ± 2.47^{a}	31.52 ± 2.65^{b}	39.32 ± 1.04^{ab}	62.72 ± 1.28^{c}
		Over all mean	44.81 ± 5.28^{A}	27.85 ± 4.06^{B}	25.52 ± 1.78^{B}	35.54 ± 2.33^{C}	56.81 ± 6.58^{D}

Different superscripts (a, b, c, d) in the same column indicate significant difference amongst different recovery periods (Duncan's multiple range test, $\alpha=0.05$). Different superscripts (A, B, C, D) in the same row indicate significant difference ($p<0.05$) (overall mean values) between different treatments (control, T_1, T_2, T_3 and T_4). Values are expressed as mean ± SE ($n=4$). Units: mg/100 ml hemolymph (glucose), mg/g wet hepatopancreas (glycogen), μg/g wet tissue (ascorbic acid).

Table 5
Gluconeogenic enzymes of *M. rosenbergii* in response to claw ablation and mitigation using high protein and vitamin C diet

		Recovery period	Control	T_1	T_2	T_3	T_4
G6Pase							
	hepatopancreas	0 h	5.68 ± 0.67^{a}	9.62 ± 0.82^{a}	9.57 ± 1.43^{ab}	11.6 ± 7.59	8.3 ± 1.03^{ab}
		6 h	3.83 ± 0.69^{a}	13.6 ± 2.2^{b}	9.15 ± 1.35^{abc}	10.78 ± 2.51	11.1 ± 1.08^{b}
		24 h	5.11 ± 0.44^{a}	14.7 ± 0.87^{b}	12.04 ± 1.07^{bc}	12.03 ± 1.71	8.51 ± 1.66^{ab}
		7 days	5.88 ± 0.68^{a}	7.13 ± 0.81^{a}	5.48 ± 0.42^{a}	5.25 ± 0.52	5.39 ± 0.36^{a}
		14 days	12.33 ± 1.68^{b}	9.78 ± 0.81^{a}	13.66 ± 1.92^{c}	11.86 ± 1.07	10.16 ± 0.88^{b}
		Over all mean	6.57 ± 0.78^{A}	10.96 ± 0.8^{B}	9.97 ± 0.83^{B}	10.3 ± 1.58^{B}	8.69 ± 0.62^{AB}
FBPase							
	hepatopancreas	0 h	3.88 ± 0.35^{a}	7.11 ± 0.31^{a}	7.93 ± 0.61^{ab}	7.22 ± 0.35^{ab}	8.02 ± 0.28^{a}
		6 h	4.45 ± 0.21^{a}	8.98 ± 0.41^{b}	9.04 ± 0.74^{b}	4.82 ± 0.34^{c}	7.91 ± 0.24^{a}
		24 h	3.97 ± 0.35^{a}	6.9 ± 0.38^{a}	4.44 ± 0.43^{c}	5.91 ± 0.56^{bc}	5.95 ± 0.31^{b}
		7 days	4.06 ± 0.42^{a}	3.69 ± 0.43^{c}	3.59 ± 0.43^{c}	4.51 ± 0.63^{c}	4.25 ± 0.06^{c}
		14 days	6.36 ± 0.34^{b}	7.51 ± 0.68^{a}	6.44 ± 0.73^{a}	8.74 ± 0.61^{a}	7.87 ± 0.82^{a}
		Over all mean	4.55 ± 0.24^{A}	6.83 ± 0.43^{B}	6.29 ± 0.53^{B}	6.24 ± 0.41^{B}	6.81 ± 0.38^{B}

Different superscripts (a, b, c) in the same column indicate significant difference amongst different recovery periods (Duncan's multiple range test, $\alpha = 0.05$). Different superscripts (A, B) in the same row indicate significant difference ($p < 0.05$) (overall mean values) between different treatments (control, T_1, T_2, T_3 and T_4). Values are expressed as mean ± SE ($n = 4$). Units: μg phosphorus released/mg protein/min at 37 °C (G6Pase and FBPase).

evident in the hepatopancreas up to 24 h after claw ablation ($p < 0.05$). Increased LDH levels in the muscle were evident in all treatment groups (T_1 to T_4) over the controls irrespective of the recovery period. A decrease in ALT activity was evident in the hepatopancreas up to 6 h after claw ablation ($p < 0.05$). However, a significantly ($p < 0.05$) higher ALT activity was evident in all treatment groups T_1, to T_4 over control irrespective of the recovery period.

4. Discussion

The main aim of the present study was to assess the ameliorative effect of stress due to claw ablation in *M. rosenbergii* males. Results of the proximate analysis indicate that carcass crude protein increased due to dietary high protein supplementation.

Stress of any kind leads to hyperglycemia under the influence of crustacean hyperglycemic hormone in crustaceans. However, the assay of glucose is simpler over either crustacean hyperglycemic hormone in shellfishes. Glucose continues to increase for a longer period after the onset of stress (Strange, 1980) to cope up with the energy demand due to stress (Barton and Schreck, 1987a,b; Vijayan and Moon, 1994). In the present study, metabolic responses of *M. rosenbergii* to claw ablation were tested and mitigation of stress was tested using high protein and vitamin C diet. Higher glucose levels were depicted in all treatment groups (T_1 to T_4) immediately after claw ablation (Table 4), which indicates that claw ablation induces stress to *M. rosenbergii*. Recovery from claw ablation is evident from the decreasing glucose level up to 24 h. Glycogen reserves in different treatment groups (Table 4) must have derived from gluconeogenic pathway (Table 5) utilizing substrates by enhanced transamination of alanine (Table 6).

Table 6
Lactate Dehydrogenase (LDH) and Alanine amino transferase (ALT) in hepatopancreas of *M. rosenbergii* in response to claw ablation and mitigation using high protein and vitamin C diet

	Recovery period	Control	T_1	T_2	T_3	T_4
LDH	0 h	0.31 ± 0.02^{a}	1.73 ± 0.14^{a}	1.5 ± 0.12^{a}	1.65 ± 0.17^{a}	0.87 ± 0.04^{a}
	6 h	0.52 ± 0.03^{b}	0.87 ± 0.12^{bc}	0.76 ± 0.12^{b}	0.38 ± 0.1^{c}	0.38 ± 0.04^{b}
	24 h	0.55 ± 0.07^{b}	0.42 ± 0.08^{c}	0.46 ± 0.03^{b}	0.37 ± 0.009^{c}	0.29 ± 0.02^{c}
	7 days	0.49 ± 0.05^{b}	0.63 ± 0.13^{bc}	0.5 ± 0.03^{b}	0.9 ± 0.06^{b}	0.4 ± 0.01^{b}
	14 days	0.20 ± 0.02^{a}	1.07 ± 0.22^{b}	1.32 ± 0.18^{a}	0.31 ± 0.03^{c}	0.53 ± 0.01^{d}
	Over all mean	0.42 ± 0.04^{A}	0.94 ± 0.12^{B}	0.91 ± 0.11^{B}	0.72 ± 0.12^{C}	0.49 ± 0.05^{A}
ALT	0 h	1.49 ± 0.21^{a}	4.72 ± 0.4^{a}	4.19 ± 0.26^{a}	3.15 ± 0.48	3.54 ± 0.25^{a}
	6 h	1.44 ± 0.6^{a}	2.86 ± 0.05^{b}	2.67 ± 0.14^{b}	3.17 ± 0.14	2.65 ± 0.13^{c}
	24 h	1.91 ± 0.18^{a}	3.29 ± 0.13^{b}	3.15 ± 0.12^{bc}	3.16 ± 0.11	2.86 ± 0.15^{bc}
	7 days	2.15 ± 0.42^{a}	4.84 ± 0.43^{a}	3.37 ± 0.14^{c}	3.43 ± 0.05	2.69 ± 0.06^{bc}
	14 days	4.75 ± 0.48^{b}	3.21 ± 0.04^{b}	4.65 ± 0.17^{a}	3.49 ± 0.16	3.16 ± 0.12^{ab}
	Over all mean	2.23 ± 0.32^{A}	3.78 ± 0.22^{B}	3.61 ± 0.18^{B}	3.28 ± 0.1^{C}	2.98 ± 0.1^{C}

Different superscripts (a, b, c, d) in the same column indicate significant difference amongst different recovery periods (Duncan's multiple range test, $\alpha = 0.05$). Different superscripts (A, B, C) in the same row indicate significant difference ($p < 0.05$) (overall mean values) between different treatments (control, T_1, T_2, T_3 and T_4). Values are expressed as mean ± SE ($n = 4$). Units: micromoles of pyruvate utilized/mg protein/minute (LDH), nanomoles of pyruvate formed/mg protein/minute at 37 °C (ALT).

It is well established that the pattern of metabolic adjustment to energy metabolism in an animal may undergo changes according to variations in carbohydrate, protein, and lipid contents in the diet they are fed. However, in mammals, fishes, and carnivorous birds fed high-protein diets, the gluconeogenic capacity and activity of key gluconeogenic enzymes are high in the fed animals and decrease during food deprivation (Cowey et al., 1977; Veiga et al., 1978; Kettelhut et al., 1980; Moon, 1988). The hepatopancreas of decapods has long been thought to function not only as a site for secretion of digestive enzymes, but also as a centre for carbohydrate metabolism (Hill et al., 1991). It has been suggested that the hepatopancreas might be the site of gluconeogenesis (Munday and Poat, 1971). In contrast to vertebrates (Cowey et al., 1977; Veiga et al., 1978; Kettelhut et al., 1980; Moon, 1988), intrinsic capacity of synthesis of glucose from alanine (Guendalina and Roselis, 1997) and lactic acid (Thabhrew et al., 1971; Phillips et al., 1977; Gade and Grieshaber, 1986; Aardt and Van, 1988; Henry et al., 1994) is well demonstrated in various crustaceans. In the present study, higher protein metabolism and utilization of alanine is evident from high ALT activity, which are being used for de novo synthesis of protein at the site of claw regeneration. On the other hand, keto acids are being formed by gluconeogenic pathway to supply energy for anabolic processes. Increase in lactate dehydrogenase activity was recorded (Table 6), which must be due to anaerobic metabolism, under the influence of stress due to claw ablation. A reduction in LDH activity after 24 h is a clear evidence of regaining homeostasis. In contrast to other invertebrates, crustaceans utilize only one basic pathway of anaerobic glycolysis by fermentation of glycogen into lactate (Bridges and Brand, 1980; Spotts, 1983; Aardt and Wolmarans, 1987). Therefore, the differentiated gluconeogenic capacities for lactic acid and alanine found in our study may represent a metabolic adaptive mechanism to cope up with stress and more effective gluconeogenesis from lactate was evident during severe stress immediately after claw ablation.

Our results indicate that high protein diet enriches the amino acid pool in the cells and nonessential amino acids and act to produce substrate for gluconeogenesis, which aid in combating the stress due to claw ablation. Similar results were reported when *Channa punctatus* were supplemented with high protein in presence of stress due to endosulfan (Sarma, 2004). Tissue regeneration must have been augmented after homeostasis is regained (after 24 h) as evidenced by the decrease in glucose level in 24 h. It is reported that G6Pase increases when monosaccharide levels decrease in the cell (Lahnsteiner and Patarnello, 2004). Monosaccharides are required for the synthesis of nucleic acids whose levels significantly increase during all anabolic processes (Lahnsteiner and Patarnello, 2004). In the present study, after claw ablation, epidermal cells located on the periphery of the resulting stump enlarge and migrate. After 1–2 weeks, a papilla is formed as a result of a blastema erupting through the scab and develops into a new miniclaw, which is an anabolic process. Therefore, the present investigation indicates that high protein diet may improve the regenerative capacity of ablated claws of *M. rosenbergii*.

Most aquatic animals including shrimps require a dietary source of vitamin C to prevent the development of deficiency symptoms; such as melanized lesions throughout the collagenous tissue underlying the exoskeleton, reduced growth, poor wound healing and eventually mortality (Hunter et al., 1979; Shiau and Hsu, 1994). Fish and crustaceans are incapable of biosynthesis of ascorbate since they lack L-gulonolactone oxidase, the enzyme responsible for synthesis of vitamin C (Wilson, 1973). Dietary supplementation of vitamin C is therefore essential for normal growth and body physiology of prawns due to its antioxidant property. In the present study, a significant decrease in ascorbate levels was observed until 7 days. In crustaceans, vitamin C influences the mechanism of synthesis of chitin (Paul Raj, 1997) Therefore, we hypothese that a reduction in vitamin C towards the end of recovery period (7 days) in our study must also be due to moulting and resorption of chitin. However, more research needs to be conducted to establish this hypothesis.

Overall results indicate that high protein and vitamin C supplementation may improve the regenerative capacity of ablated claws in *M. rosenbergii* males. However, claw ablation is an advisable procedure in monosex culture systems as it reduces differential growth rate and cannibalistic behavior of dominant blue-clawed males of *M. rosenbergii*. Claw ablation procedures also increase the dressing yield of *M.rosenbergii* during processing. An increase in yield, uniformity in harvesting prawns and high market value is expected by implementing this procedure in prawn culture systems.

Acknowledgements

The first author is thankful to the Indian Council of Agricultural Research for providing financial assistance in the form of an Institutional fellowship during the research period.

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