Indian Journal of Animal Sciences **82** (6): 654–659, June 2012

Dietary administration of marine oil on growth and enzymatic activity of *Macrobrachium rosenbergii*

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Received: 30 July 2011; Accepted: 23 November 2011

ABSTRACT

Feeding trial was conducted to study the efficiency of the dietary administration of marine oil on survival, growth and enzymatic activity of freshwater prawn, *Macrobrachium rosenbergi* for 112 days. Five test diets were formulated using the locally available feed ingredients namely, fish meal, groundnut cake, rice bran, tapioca flour and vitamin mineral premix. Diets contained 35% crude protein with graded lipid levels. Sardine oil was incorporated at 4 levels and the corresponding total lipid levels were 8.11, 10.24, 12.28 and 14.33%, while diet without sardine oil (6.05% total lipid) served as control. Significantly higher weight gain of prawn was recorded in the treatment with 6.07% sardine oil (12.28% total lipid). Growth parameters such as SGR, FCR and PER were better for the same dietary treatment. The highest RNA/DNA ratio in the muscle of prawn was recorded in the treatment which had 8.07% sardine oil (14.33% total lipid), which is significantly different from other diets, except 6.07% sardine oil treatment. Digestive enzyme analysis in the digestive tract and mid-gut gland of *M. rosenbergii* showed greater activity in 8.07% dietary treatment.

Key words : Diet, Enzymes, Growth, *Macrobrachium rosenbergii*, Nucleic acids, Sardine oil

Freshwater prawn, *Macrobrachium rosenbergii* is farmed in inland water bodies on large scale. It is also popularly called as scampy in the trade, and is the most preferred species for culture in freshwaters in many parts of the world (New 1995, 2000). Enzymatic studies have confirmed the omnivorous feeding habit of *M. rosenbergii* (Lee *et al.* 1980, 1984). This species normally feeds on aquatic insects, larvae, algae, nuts, grains, seeds, fruits, small crustaceans, fish flesh, offal and other animal feeds in nature (Prasad 1996), but readily accepts pellet feeds. Dietary protein level of *M. rosenbergii* is between 23 and 50%, however 14% dietary protein also yielded satisfactory growth (Bartlett and Enkerlin 1983) in the presence of natural food. A dietary lipid level of 6–8% was satisfactory for juvenile *M. rosenbergii*, and growth was poor on a lipid-free diet (Sheen and D'Abramo 1991). Freshwater prawns appear to require polyunsaturated fatty acids of C18 and C20 series as its capability to synthesize HUFAs from PUFAS is limited (D'Abramo and Sheen 1993, Reigh and Stickney 1989). The present study was conducted to study the effect of graded level dietary administration of sardine oil on growth, survival, biochemical and enzymatic changes in *M. rosenbergii*.

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MATERIALS AND METHODS

Experimental diets: Sardine (*Sardinella* sp.) oil, used as a source of marine lipid, was incorporated at 2.07, 4.07, 6.07 and 8.07% in 5 test diets, viz. D8, D10, D12 and D14 respectively and diet D6 served as control. The remaining portion of dietary lipid comes from other feed ingredients used and subsequently total lipid levels in the prepared diets found to be 6, 8, 10, 12 and 14% (dry weight) in D6, D8, D10, D12 and D14 respectively. A constant dietary protein level (35%) was maintained in all the diets (Table 1).

Chemical analysis: A proximate analysis of formulated diets was carried out (AOAC 1995). The difference method of Hastings (1976) to estimate carbohydrate content as nitrogen-free extract (NFE) and energy levels of the feeds as kJ g^{-1} were estimated using bomb calorimeter (Table 1). Protein to energy ratio (P: E) and energy to protein ratios (E: P) for the formulated feeds were calculated (Steffens 1989).

Rearing system: Uniform-sized outdoor cement cisterns (15) (measuring 5 m ×5 m ×1m) were used for the experiment. The cisterns were filled with filtered freshwater drawn from a nearby open well. The water level in the cisterns was maintained at 60±5 cm throughout the experimental period. Evaporation loss was compensated by replenishing

freshwater once in a week.

Experimental animals: M. rosenbergii post-larvae produced in the hatchery of the College of Fisheries, Mangalore, India were reared up to juvenile stage in nursery tanks. During this period, the prawn post larvae were fed with a practical diet containing 30% protein. Juveniles were stocked in rearing tanks in 3 replicated groups with stocking density of 50 animals per tank. Aquatic floating weed, *Eichhornia* plants were spread on the water surface covering nearly 1/10th of each of the tank surface as shelter for the prawns, in addition to this PVC pipes of 30 cm length and 10 cm diameter were also spread on the pond bottom covering 4% of the tank bottom area uniformly in all the tanks.

Feeding: The animals were fed with a basal diet containing 35% protein @ 10% of the body weight in 2 equal meals for first 30 days, and thereafter feeding rate was reduced to 5% for the remaining period and feeding trial was carried out for 112 days.

Sampling

Prawn: Prawns were measured for weight and length on the day of stocking and sampling was carried at every fortnight during the feeding trial. At the end of the experiment, tanks were drained and prawns were measured for final individual length and weight.

Water: Water quality maintained in the rearing tanks throughout the experimental period. The evaporation loss was compensated by filling with freshwater every week. Water sampling was carried out once every week and the collected samples were measured for pH, temperature by using water quality analyzer, while Winkler's method was used to estimate dissolved oxygen. Free carbon dioxide, total alkalinity and ammonia-nitrogen were estimated as per standard methods (APHA 1998).

Biochemical analysis: Before starting of the experiment and at the end proximate composition of prawn muscle was analyzed. The muscle was dried at 60°C for 12 h to obtain dry matter. The dry matter was powdered using pestle and motor and stored in air-tight polythene covers for further analyses. The samples were analyzed for crude protein, crude fat, total ash and carbohydrate (as nitrogen-free extract) and energy content by following methods mentioned earlier under analysis of feeds.

Analysis of nucleic acids: The prawn muscle nucleic acids (RNA and DNA) were extracted using perchloric acid (Burton 1956). DNA content was determined by diphenylamine method (Giles and Myres 1965) and RNA as per Ceriotti (1955).

Digestive enzymes: Both digestive tract and midgut gland of prawn from each treatment were taken for the analysis of digestive enzymes. Total activity of protease was measured by the casein digestion method (Kunitz 1947). Amylase activity (amylase) was evaluated through reduction of 3–5, dinitro-salicylic acid by the reducing groups liberated from starch during enzyme hydrolysis (Sumner and Somers 1947). Titrimetric method (Bier 1962) was used for the estimation of lipase activity. The method of Lowry *et al.* (1951) was employed for the estimation of protein in crude enzyme extract.

Statistical analysis: Significant difference among the means of different dietary treatments on prawn growth, proximate composition of prawn muscle, nucleic acid levels and digestive enzyme activities using Analysis of variance (Snedecor and Cochran 1968). Further, Duncan's multiple range test (Duncan 1955) was used to test significant differences ($P < 0.05$).

Table 1. Proximate composition (%)* of the experimental diets

Particulars	Diets				
	D_6	D_8	D_{10}	D_{12}	D_{14}
Dry matter	91.93(0.17)	91.50(0.10)	91.86(0.03)	91.56(0.06)	91.74(0.05)
Crude protein	35.31^{ab}(0.09)	35.54^{c}(0.05)	35.46^{c}(0.06)	35.50^{c}(0.09)	35.20^{ab}(0.02)
Crude fat	6.05^{a}	8.11^{b}	10.24^{c}	12.28^{d}	14.33^{e}
Sardine oil incorporated %	0	2.07	4.07	6.07	8.07
% of total lipid	0	25.52	39.75	49.43	56.32
Crude fiber	7.30^{a}(0.12)	7.62^{ab}(0.06)	7.59^{ab}(0.04)	7.44^{ab}(0.05)	7.80^{b}(0.10)
Ash	13.45(0.03)	13.37(0.14)	13.40(0.11)	13.25(0.16)	13.36(0.10)
Carbohydrate (NFE)	29.82^{e}(0.21)	26.86^{d}(0.07)	25.17^{c}(0.15)	23.09^{b}(0.10)	21.05^{a}(0.19)
Energy (kJ g-1)	14.68^{a}(0.03)	15.00^{b}(0.01)	15.51^{c}(0.04)	15.94^{d}(0.04)	16.29^{e}(0.02)
P/E ratio (mg protein/kJ/g)4	24.04^{e}(0.06)	23.69^{d}(0.04)	22.87^{c}(0.05)	22.27^{b}(0.06)	21.61^{a}(0.04)
E/P ratio5	4.16^{a}(0.01)	4.22^{b}(0.01)	4.37^{c}(0.01)	4.49^{d}(0.01)	4.63^{e}(0.01)

*Different superscripts (a, b, c, d) in the same row indicate significant difference ($P<0.05$) among the control and treatment groups (Duncan's multiple range test, a=0.05). Values are expressed as mean±SE (n=6).

$$\text{P/E ratio} = \frac{\text{Protein content of the diet (mg kg-1)}}{\text{Energy content of the diet kJ g-1)}}$$

$$\text{E/P ratio} = \frac{\text{Energy content of the diet kJ g-1)}}{\text{Crude proteinn content (\%)}}$$

RESULTS AND DISCUSSION

The best growth (weight and length) of prawn was observed in D12, which had 12.28% total lipid and 6.07% sardine oil. Higher survival of prawn was recorded in D12 which did not differ significantly from that of D10 (Table 2). Higher SGR of prawn was recorded in diet D12. Best FCR was recorded in treatment D12 (2.31) and it did not differ significantly from that of the other diets. Similarly, PER was also did not differ significantly ($P > 0.05$) among the dietary treatments.

Biochemical composition of prawn muscle indicated that crude fat and moisture in the prawn muscle decreased at the end of the experiment. The crude protein, ash, NFE and energy levels in the prawn muscle showed an increased trend at the end of experiment (Table 2).

Significantly higher RNA and DNA levels were observed in prawn fed diet D12 and did not differ significantly between D12 and D10 treatments (Table 3). The higher total activity and the specific enzyme activity of protease, amylase and lipase were recorded in digestive tract and hepatopancreas of prawn in all the treatments, while the lowest values were recorded in D6 (Table 4). The range of water quality parameters recorded were, pH 7.20–8.10; temperature 27–29.50°C; dissolved oxygen 6.5–10.6 mg/l; free carbon dioxide 0– 2.4 mg/l, total alkalinity 72.60–87.50 mg/l and ammonia-nitrogen 1.36–8.75 ng/l. Water quality parameters recorded during the experiment were found suitable for the prawn growth.

Prawns were fed with a diets containing sardine oil had higher growth than that of control diet. The diet D12 resulted in best growth. However, there was no significant difference between groups fed D10, D12 and D14 diets, clearly indicating that sardine oil induced a positive response on the growth of prawn. A wide range of dietary lipid levels was evaluated for juvenile *M. rosenbergii,* but growth was poor on a lipid free diet (Sheen and D'Abramo 1991). It was reported that 6% dietary lipid, comprising pollack liver oil and soybean oil in 1:1 ratio, was found optimal for juvenile *Penaeus japonicus* (Kanazawa *et al.* 1977). They also reported that 8% lipid from short neck clam in a casein based diet indicated positive response in *P. japonicus* in comparison

Table 2. Growth and proximate composition of muscle of *M. rosenbergii*

Particulars	Diets				
	D_6	D_8	D_{10}	D_{12}	D_{14}
Dry					
Initial weight[1] (g)	1.21±0.01	1.12±0.02	1.31±0.01	1.11±0.01	1.22±0.02
Final weight[1] (g)	15.51±1.31^{a}	19.76±1.32^{ab}	26.23±0.93^{bc}	28.91±1.07^{c}	25.10±1.59^{bc}
Initial length (cm)	3.00±0.16^{b}	2.51±0.07^{a}	3.55±0.07^{c}	2.60±0.01^{a}	3.57±0.16^{c}
Final length (cm)	8.74±0.21^{a}	10.37±0.38^{b}	12.16±0.42^{c}	15.26±0.43^{d}	11.45±0.15^{bc}
Weight gain[1] (g)	14.31±1.33^{a}	18.65±1.34^{ab}	24.92±0.94^{bc}	27.81±1.07^{c}	23.89±1.58^{bc}
% Weight gain	1181.80 ^{a}	1664.30 ^{ab}	1902.30 ^{bc}	2504.50^{c}	1957.30 ^{bc}
Survival (%)	53.75±5.35^{b}	58.75±5.68^{b}	76.25±6.25^{c}	82.50±7.50^{c}	59.00±5.00^{a}
SGR	2.28±0.07^{a}	2.56±0.07^{b}	2.68±0.04^{bc}	2.91±0.03^{c}	2.70±0.05^{bc}
FCR	2.90±0.22^{a}	2.65±0.17^{a}	2.45±0.15^{a}	2.31±0.05^{a}	2.44±0.12^{a}
PER	0.98±0.01	1.07±0.07	1.15±0.07	1.22±0.03	1.17±0.07

Expressed on wet weight basis

$$SGR = \frac{\ln (Wt_2) - \ln (Wt_1)}{t_2 - t_1} \times 100$$

where, ln (Wt1) = natural log of the weight of animals at time 1 (t_1); ln (Wt_2) = natural log of the weight of animals at time 2 (t2)

Proximate composition (%) of prawn muscle

Parameter	Initial analysis	Final analysis				
Moisture	86.65±0.16^{b}	81.78±0.28^{a}	80.72±0.25^{a}	81.09±0.44^{a}	81.80±0.44^{a}	81.52±0.19^{a}
Crude protein	11.26±0.05^{a}	14.45±0.28^{b}	14.65±0.17^{b}	14.57±0.16^{b}	14.14±0.08^{b}	14.66±0.17^{b}
Crude fat	0.93±0.03^{c}	0.59±0.03^{a}	0.65±0.01^{a}	0.79±0.02^{b}	0.90±0.02^{bc}	0.82±0.03^{b}
Ash	0.10±0.03^{a}	1.33±0.05^{c}	1.50±0.08^{d}	1.95±0.02^{d}	1.06±0.03^{b}	1.92±0.01^{d}
NFE	1.05±0.18^{a}	1.85±0.47^{b}	2.49±0.33^{d}	1.60±0.19^{b}	2.09±0.39^{c}	1.08±0.13^{a}
Energy (kJ g^{-1})	2.48±0.03^{a}	3.03±0.05^{b}	3.20±0.04^{b}	3.09±0.07^{b}	3.14±0.08^{b}	3.02±0.03^{b}

Different superscripts (a, b, c, d) in the same row indicate significant difference ($P<0.05$) among the control and treatment groups (Duncan's multiple range test, $P=0.05$). Values are expressed as mean±SE (n,6).

Table 3. RNA, DNA levels and RNA/DNA ratio in the muscle of *M. rosenbergii*

Diets	RNA(mg/g)	DNA(mg/g)	RNA/DNA ratio
D_6	6.22±0.15[a]	2.16±0.06[a]	2.83±0.05[b]
D_8	7.05±0.06[a]	3.80±0.08[c]	1.86±0.02[a]
D_{10}	10.53±0.84[b]	3.74±0.08[c]	2.82±0.21[b]
D_{12}	14.42±0.73[c]	4.67±0.11[d]	3.08±0.13[bc]
D_{14}	10.07±0.22[b]	2.93±0.15[b]	3.45±0.13[c]

Different superscripts (a, b, c, d) in the same column indicate significant difference (P<0.05) among the control and treatment groups (Duncan's multiple range test, a=0.05). Values are expressed as mean±SE (n,6).

to purified diets. It is demonstrated from the present study and in the light of published reports that juvenile *M. rosenbergii* grows well when fed diets that contained 6.07% of sardine oil. The growth of prawns and shrimps is affected by the fatty acid composition of the diet, its protein to lipid ratio, protein to carbohydrate ratio and to some extent the lipid classes present in the feed (Fox *et al.* 1994). However, no study has reported any beneficial effect of lipid at 15% or more. The inability of prawns and shrimp to utilize high levels of dietary lipid probably indicates low lipid levels in their natural diet (Dall *et al.* 1991) and low lipid levels found in their tissues (Fox *et al.* 1994). Better growth of prawn fed diets containing sardine oil is attributed to presence of highly unsaturated fatty acids (HUFAs) particularly, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), because freshwater prawn require these fatty acids in their diet (Murthy and Thanuja 2005).

Survival is equally important to achieve high production in grow-out ponds. Both survival and growth depend upon feed utilization, which is influenced by several factors such as availability and the type of feed, physico-chemical characteristics of water and physiological conditions of the cultured animals. In the present study, *M. rosenbergii* recorded a highest survival (82.5%) in D12 and lowest in D6 (53.75%). Similar results were reported earlier indicating low survival of prawn with low level of incorporated marine oil (Bazaz and Keshavanath (1993). Specific growth rate (SGR) is an index of growth in assessing the quality of diet. Higher SGR indicates better utilization and efficient conversion of the feed by prawn. Several workers have reported the differential growth (heterogeneous individual growth) of *M. rosenbergii* in the normal bi-sex population in grow out farming (Mathew and Mohan 1994, Murthy 1998). However, the differential growth was not prominent in the present study, probably due to the provision of shelters in the tanks.

Water temperature, size of the animal, stocking rate, quality of feed and availability of natural food influence feed conversion. There was no significant difference among the treatments with respect to FCR in the present study. The recorded FCR and protein efficiency ratio (PER) values were satisfactory and comparable to the findings of earlier workers (Naik and Murthy 1999, Srinivasa *et al.* 2003). The RNA/DNA ratio serves as an index of growth per cell with higher ratios indicating growth enhancement (Sable 1974). It is reported that the ratio is a sensitive index of nutrition, where the lowest values were obtained in response to starvation and conversely high values were indicative of good nutrition and rapid growth (Wright and Martin 1985, Clemmenson 1987). In this study, highest RNA/DNA value was recorded in prawn fed diet D14, which did not differ significantly from that of treatment D12, where the growth of prawn was

Table 4. Protease, amylase and lipase activity [total activity (T.A) and specific activity (Sp.A)] in the digestive tract and mid-gut gland of *M. rosenbergii*

Diet	Activity	Protease		Amylase		Lipase	
		Digestive tract	Mid-gut gland	Digestive tract	Mid-gut gland	Digestive tract	Mid-gut gland
D_6	T.A	45.23±0.78[a]	25.17±0.83[a]	121.53±1.08[a]	75.24±0.95[a]	0.238±0.001[a]	0.721±0.02[a]
D_8	T.A	51.67±0.97[b]	27.75±0.66[a]	146.95±1.00[b]	82.21±0.82[b]	0.334±0.001[b]	0.89±0.012[b]
D_{10}	T.A	64.48±0.66[c]	33.45±0.83[b]	154.96±0.86[c]	94.90±0.90[c]	0.553±0.002[c]	1.006±0.07[c]
D_{12}	T.A	77.35±0.74[d]	39.34±0.36[c]	159.30±1.06[d]	101.21±0.99[d]	0.910±0.001[e]	1.210±0.018[d]
D_{14}	T.A	62.88±1.07[c]	35.69±0.95[b]	151.74±0.91[bc]	91.56±1.06[c]	0.598±0.008[d]	0.999±0.009[c]
D_6	Sp.A	0.683±0.012[a]	0.672±0.022[a]	1.834±0.016[a]	2.008±0.025[a]	0.004±0.00[a]	0.019±0.001[a]
D_8	Sp.A	0.725±0.014[a]	0.704±0.01[a]	2.063±0.014[b]	2.066±0.35[b]	0,005±0.00[a]	0.023±0.00[b]
D_{10}	Sp.A	0.905±0.009[b]	0.801±0.02[b]	2.176±0.01[c]	2.274±0.02[c]	0.008±0.001[b]	0.024±0.00[b]
D_{12}	Sp.A	1.066±0.01[c]	0.904±0.008[c]	2.196±0.015[c]	2.325±0.023[c]	0.013±0.00[d]	0.028±0.000[c]
D_{14}	Sp.A	0.914±0.01[b]	0.877±0.023[bc]	2.205±0.013[c]	2.249±0.026[c]	0.009±0.00[c]	0.025±0.00[b]

Different superscript (a, b, c, d) in the same column indicate significant difference (P<0.05) among the control and treatment groups (Duncan's multiple range test, P=0.05). Values are expressed as mean±SE (n=6).

TA, Total activity (enzyme units/g tissue); SpA, Specific activity (enzyme units/mg protein) Enzyme units, N moles of product liberated/ gram of tissue/10 min at 30°C.

highest. This indicates an increase in protein synthesis probablyresulted in higher growth of freshwater prawn (Srinivasa 2003).

Digestive enzymes (protease, amylase and lipase) play a very important role in the digestibility of feed (Dabrowski and Glogowski 1977). All the digestive enzymes originate in the midgut gland (referred as hepatopancreas), where a number of metabolic activities in decapod crustaceans occur (Ceccaldi 1997). Several studies have indicated that the relative activity of the digestive enzymes could be correlated with the nature and composition of the food consumed (Kawai and Ikeda 1972, Olatunde and Ogunbiyi 1977). Variation in the activities of the three enzymes recorded in the present study could be due to varied levels of sardine oil incorporated in the diets. The protein accretion was comparatively higher in the digestive tract than in mid-gut gland. Diets employed in the present study had different levels of sardine oil and the highest protease activity (total and specific activity) was recorded in the treatment D12, which probably could be due to presence of higher levels of essential fatty acids. Protease activity was higher in the intestine of carps (common carp, catla and silver carp) when fed a protein rich diet (Phadate 1987). Further, Kawai and Ikeda (1972) and Shcherbina *et al*. (1976) reported adaptive changes in the activity of proteolytic enzymes in relation to the type of diet in common carp. The changes in proteolytic activities of prawn among different dietary treatments in which fish meal and silage based diets were used were not statistically different (Ali *et al.* 2000). Amylase, one of the most important carbohydrases, showed The generally high activity, but there was a significant variation in its activity among the diets. Feeding habit of the animal and type of food has an influence on amylase activity (Phadate 1987). Amylase activity (total and specific activity) was significantly higher in the digestive tract as well as in mid-gut gland of *M. rosenbergii,* fed diets containing sardine oil compared to the marine oil free diet. Similar observations were recorded in *M. rosenbergii* fed probiotic incorporated diets (Ali *et al.* 2000, Srinivasa *et al*. 2003). Lipase activity in the present study was higher in hepatopancreas than in digestive tract. Higher lipase activity was found in the mid-gut gland of carp fingerlings (Das and Tripathi 1991,Srinivasa *et al.* 2003). Lee *et al.* (1984) observed that protein source had a greater effect on the enzyme activities.

Deterioration in the quality of water in aquaculture systems is usually associated with accumulation and decomposition of organic matter. However, the water quality recorded in the present study was within the suitable range for prawn culture (Naik and Murthy 1999, Naik *et al.* 2000) and managed by partial replacement of water once in every week.

Higher levels of highly unsaturated fatty acids (EPA and DHA) are available in marine sardine oil. Growth and survival in *M. rosenbergii* enhanced when incorporated it as a lipid source in the diets. Nucleic acid composition and enzyme profile of prawn was found improved by feeding diets, which contained sardine oil.

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