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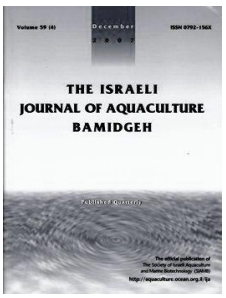

Effect of Dietary Administration of Sardine Oil on Growth, Survival, and Enzymatic activity of *Macrobrachium rosenbergi*i (de Man)

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Key words: *Macrobrachium rosenbergii*, diet, sardine oil, growth, enzymes, nucleic acids

Abstract

Five test diets were formulated using locally available feed ingredients: fishmeal, groundnut cake, rice bran, tapioca flour, and vitamin/mineral premix. Diets contained 35% crude protein with graded lipid levels. Sardine oil was incorporated at four dietary levels: 2.07%, 4.07%, 6.07%, or 8.07%, with corresponding total lipid levels of 8.11%, 10.24%, 12.28%, and 14.33%. A diet without sardine oil (6.05% total lipid) served as control. The diets were fed to giant freshwater prawns, *Macrobrachium rosenbergii*, for 112 days. A significantly higher weight gain was recorded in the treatment with 6.07% sardine oil and the specific growth rate, food conversion rate, and protein efficiency ratio were best in this treatment. The diet containing 8.07% sardine oil produced the highest RNA:DNA ratio in the prawn muscle, significantly similar to the ratio in prawns fed the 6.07% diet and significantly higher than in prawns fed the other diets. Digestive enzyme analyses in the digestive tract and midgut gland showed the greatest activity in prawns fed the 8.07% diet.

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Introduction

Freshwater prawns are farmed in inland water bodies on a commercial scale and, since they are tolerant to salinity fluctuation, in low saline brackish waters. Among species of the genus *Macrobrachium*, the giant freshwater prawn, *M. rosenbergii*, is suitable for farming because of its fast growth. It is popularly called 'scampy' and is the most preferred species for freshwater culture in many parts of the world (New, 2000).

The omnivorous feeding habits of *M. rosenbergii* were confirmed by enzyme studies (Lee et al., 1984). In nature, *M. rosenbergii* feeds on aquatic insects, larvae, algae, nuts, grains, seeds, fruits, small crustaceans, fish flesh, offal, and other animal feeds, but it readily accepts pelleted feeds. The required dietary protein level for *M. rosenbergii* is 23-50%. However, 14% dietary protein has yielded satisfactory growth in the presence of natural food (Bartlett and Enkerlin, 1983). A dietary lipid level of 6-8% is satisfactory for juvenile *M. rosenbergii*, but growth is poor on lipid-free diets (Sheen and D'Abramo, 1991).

Freshwater prawns appear to require polyunsaturated fatty acids (PUFA) of the C18 and C20 series and they are capable of synthesizing highly unsaturated fatty acids (HUFA) from PUFA, i.e., 20:5 n-3 and 20:4 n-6 from 18:3 n-3 and 18:2 n-6, respectively (D'Abramo and Sheen, 1993). Oil of the sardine, *Sardinella logiceps*, is produced locally, rich in PUFA (omega-3 fatty acids such as eicosa pentaenoic acid [EPA] and docosa hexaenoic acid [DHA]), and accepted through feed by the aquacultured animal. Hence, in this study, we evaluate the effect of graded levels of sardine oil, a marine lipid, on growth, survival, and biochemical and enzymatic changes in *M. rosenbergii*.

Materials and Methods

Experimental diets. Five test diets were prepared incorporating different lipid levels (Table 1). Sardine oil (*Sardinella sp.*) was used as a source of lipid. The remaining dietary lipid came from other feed ingredients. Dietary protein was 35% in all diets. Proximate analysis of feed ingredients and formulated diets was carried out according to

Table 1. Ingredients and proximate compositions (n = 3, dry weight basis) of the experimental diets.

	Diet (sardine oil supplementation)				
	0%	*2.07%*	*4.07%*	*6.07%*	*8.07%*
Ingredient (%)					
Fishmeal[1]	33	33	33	33	33
Groundnut cake	35	35	35	35	35
Rice bran	22	22	22	22	22
Tapioca flour	9	9	9	9	9
Vitamin/mineral premix[2]	1	1	1	1	1
Sardine oil (% of total lipid)[3]	0	2.07	4.07	6.07	8.07
Proximate composition (%)					
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±0.09	35.54±0.05	35.46±0.06	35.50±0.09	35.20±0.02
Crude fat	6.05±0.05	8.11±0.02	10.24±0.05	12.28±0.10	14.33±0.04
Crude fiber	7.30±0.12	7.62±0.06	7.59±0.04	7.44±0.05	7.80±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±0.21	26.86±0.07	25.17±0.15	23.09±0.10	21.05±0.19
Energy (kJ/g)	14.68±0.03	15.00±0.01	15.51±0.04	15.94±0.04	16.29±0.02
P/E ratio (mg protein/kJ)[4]	24.04±0.06	23.69±0.04	22.87±0.05	22.27±0.06	21.61±0.04
E/P ratio (kJ/g)[5]	4.16±0.01	4.22±0.01	4.37±0.01	4.49±0.01	4.63±0.01

[1] produced from marine pelagic fish (sardines)
[2] 1 kg Supplevite-M, as supplied: Vitamin A 2,000,000 IU, Vitamin D_3 400,000 IU, Vitamin B2 0.80 g, Vitamin E 300 units, Vitamin K 0.40 g, calcium pantothenate 1.0 g, nicotinamide 4.0 g, Vitamin B_{12} 2.4 g, choline chloride 60.0 g, calcium 300.0 g, manganese 11.0 g, iodine 4.0 g, iron 3.0 g, zinc 6.0, copper 0.80 g, cobalt 0.18 g
[3] Contains: free fatty acids = 0.16±0.12%, peroxide value = 7.18±0.82 millimoles O_2/kg fat, saponification value = 176.71±0.48, iodine value = 152.71±8.98
[4] P/E ratio = protein content of diet/energy content of diet
[5] E/P ratio = energy content of the diet in kJ/g crude protein content in %

AOAC (1995). The carbohydrate content (nitrogen-free extract) was estimated by the difference method (Hastings, 1976) and energy levels (kJ/g) were estimated using bomb calorimeter.

Rearing system. Fifteen outdoor cement cisterns (5 × 5 × 1 m) were filled with filtered fresh water from a nearby open well. The water level in the cisterns was maintained at 60±5 cm throughout the experiment. Evaporation loss was compensated by replenishing fresh water once a week. Water was sampled every fortnight and samples were measured for pH as well as temperature using a Horiba Water Quality Analyzer (U-10). Winkler's method was used to estimate dissolved oxygen. Free carbon dioxide, total alkalinity, and ammonia-nitrogen were estimated by standard methods (APHA, 1998).

Experimental animals, feeding, sampling. Postlarvae of the giant freshwater prawn, *Macrobrachium rosenbergii*, produced in the hatchery of the College of Fisheries were reared to juvenile stage in nursery tanks. During nursing, the postlarvae were fed a practical diet containing 30% protein. Juveniles were stocked in rearing tanks in three replicates at 50 animals per tank. The aquatic floating weed, *Eichhornia*, was spread on the water surface as shelter for the prawns and covered nearly 10% of the tank surface. In addition, PVC pipes (30 × 10 cm) were laid on the pond bottom, covering 4% of the bottom area. Prawns were offered a daily ration equivalent to 10% of their body weight, divided into two equal meals, for the first 30 days; thereafter, until day 112, the feeding rate was reduced to 5%. Prawns were measured for weight and length on the day of stocking and every fifteen days. At the end of the experiment, tanks were drained and prawns were measured for individual length and weight.

Biochemical analyses. The proximate composition of the prawn muscle was determined at the beginning of the experiment (before stocking the animals) and at the completion of the experiment. Muscle was dried at 60°C for 12 h to obtain dry matter which was powdered with a mortar and pestle and stored in air-tight polythene covers for further analysis of crude protein, crude fat, total ash, carbohydrate (AOAC, 1995), and energy content as described above. RNA and DNA were extracted using perchloric acid (Burton, 1956). DNA content was determined by the diphenylamine method (Giles and Myers, 1965) and RNA as described by Ceriotti (1955). The digestive tract and hepatopancreas of prawns from each treatment were taken for analysis of digestive enzymes. Total protease activity was measured by the casein digestion method (Kunitz, 1947), amylase activity (∞-amylase) through reduction of 3-5, dinitro-salicylic acid by reducing groups liberated from starch during enzyme hydrolysis (Sumner and Somers, 1947). Lipase assay was estimated by the titrimetric method (Bier, 1962) and protein in the crude enzyme extract using bovine serum albumin as the standard according to the method of Lowry et al. (1951).

Statistical analysis. Significant differences ($p<0.05$) among treatments in growth, proximate composition, nucleic acid contents, and digestive enzyme activities were tested by analysis of variance (Snedecor and Cochran, 1968) and Duncan's multiple range test (Duncan, 1955).

Results

The best growth (weight and length), survival, specific growth rate, and food conversion ratio were obtained in the group fed the 6.07% sardine oil diet (Table 2). Crude fat and moisture in the prawn muscle were lower at the end of the experiment than at the beginning while crude protein, ash, NFE, and energy increased. Also, RNA and DNA were significantly highest in prawns fed this diet. Total activity and specific enzyme activity in the digestive tract and hepatopancreas were higher in prawns fed the supplemented diets than in prawns fed the control. Water quality ranged: pH 7.20-8.10, temperature 27-29.5°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 µg/l.

Table 2. Growth, proximate composition, RNA, DNA, initial (enzyme units[1]/g tissue±SE) and final (enzyme units/mg protein±SE) protease, amylase, and lipase activity in *Macrobrachium rosenbergii* fed diets with different levels of sardine oil.

	Diet (sardine oil supplementation)				
	0%	*2.07%*	*4.07%*	*6.07%*	*8.07%*
Initial wt (g)	1.21±0.01	1.12±0.01	1.31±0.01	1.11±0.01	1.22±0.02
Final wt (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}
Wt gain (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}
Wt gain (%)	1181.8	1664.3	1902.3	2504.5	1957.3
Survival (%)	53.75±8.75^{b}	58.75±6.25^{b}	76.25±6.25^{c}	82.50±7.50^{c}	59.00±5.00^{a}
Specific growth rate[2]	2.28±0.07^{a}	2.56±0.07^{b}	2.68±0.04^{bc}	2.91±0.03^{c}	2.70b±0.05^{c}
Food conversion ratio	2.90±0.22^{a}	2.65±0.17^{b}	2.45±0.15^{a}	2.31±0.05^{a}	2.44±0.12^{a}
Protein efficiency ratio	0.98±0.08	1.07±0.07	1.15±0.07	1.22±0.03	1.17±0.07
Proximate composition of prawn muscle (dry weight basis)[3]					
Moisture (%)	81.78±0.28	80.72±0.25	81.09±0.37	81.80±0.44	81.52±0.19
Crude protein (%)	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 (%)	0.59±0.03^{a}	0.65±0.01^{a}	0.79±0.02^{b}	0.90±0.02^{bc}	0.82±0.03^{b}
Ash (%)	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 (%)	1.85±0.47	2.49±0.33	1.60±0.19	2.09±0.39	1.08±0.13
Energy (kJ/g)	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}
RNA (mg/g)	6.22±0.15^{a}	7.05±0.06^{a}	10.53±0.84^{b}	14.42±0.73^{c}	10.07±0.22^{b}
DNA (mg/g)	2.16±0.06^{a}	3.80±0.08^{c}	3.74±0.08^{c}	4.67±0.11^{d}	2.93±0.15^{b}
RNA:DNA ratio	2.83±0.05^{b}	1.86±0.02^{a}	2.82±0.21^{b}	3.08±0.13^{bc}	3.45±0.13^{c}
Protease activity					
Digestive tract, initial	45.23±0.78^{a}	51.67±0.97^{b}	64.48±0.66^{c}	77.35±0.74^{d}	62.88^{c}±1.07^{c}
Digestive tract, final	0.683±0.012^{a}	0.725±0.014^{a}	0.905±0.009^{b}	1.066±0.010^{c}	0.914±0.016^{b}
Midgut gland, initial	25.17±0.83^{a}	27.75±0.66^{a}	33.45±0.83^{b}	39.34±0.36^{c}	35.69±0.95^{b}
Midgut gland, final	0.672±0.022^{a}	0.704±0.017^{a}	0.801±0.02^{b}	0.904±0.008^{c}	0.877±0.023 ^{bc}
Amylase activity					
Digestive tract, initial	121.53±1.08^{a}	146.95±1.00^{b}	154.96±0.86^{c}	159.30±1.06^{d}	151.74±0.91^{bc}
Digestive tract, final	1.834±0.016^{a}	2.063±0.014^{b}	2.176±0.012^{c}	2.196±0.015^{c}	2.205±0.013^{c}
Midgut gland, initial	75.24±0.95^{a}	82.21±0.82^{b}	94.90±0.90^{c}	101.21±0.99^{d}	91.56±1.06^{c}
Midgut gland, final	2.008±0.025^{a}	2.066±0.35^{b}	2.274±0.022^{c}	2.325±0.023^{c}	2.249±0.026^{c}
Lipase activity					
Digestive tract, initial	0.238±0.014^{a}	0.334±0.012^{b}	0.553±0.020^{c}	0.910±0.011^{e}	0.598±0.008^{d}
Digestive tract, final	0.004±0.0^{a}	0.005±0.0^{a}	0.00±0.001^{b}	0.013±0.0^{d}	0.009±0.0^{c}
Midgut gland, initial	0.721±0.021^{a}	0.898±0.012^{b}	1.006±0.007^{c}	1.210±0.018^{d}	0.999±0.009^{c}
Midgut gland, final	0.019±0.001^{a}	0.023±0.000^{b}	0.024±0.000^{b}	0.028±0.000^{c}	0.025±0.000^{b}

Values in a row with different superscripts significantly differ (p<0.05).
[1] Enzyme unit = µ moles of product liberated per g tissue per 10 min at 30°C
[2] SGR = $(\ln_{Wt2} - \ln_{Wt1})/(t_2 - t_1) \times 100$, where $\ln_{Wt1}$ = natural log of the weight of animals at time 1 (t_1) and $\ln_{Wt2}$ = natural log of the weight of animals at time 2 (t_2)
[3] Initial (%): moisture 86.65±0.16^{b}, crude protein 11.26±0.05^{a}, crude fat 0.93±0.03^{c}, ash 0.10±0.03^{a}, NFE 1.05±0.18^{a}; energy (kJ/g) 2.48±0.03^{a}

Discussion

Prawns fed diets containing sardine oil had higher growth than those fed the control diet. The 6.07% diet yielded the best growth, but there was no significant difference between this and the 4.07% or 8.07% diets, indicating that sardine oil induced a positive response on the prawn growth. Similarly, in a study of juvenile *M. rosenbergii* fed diets containing 2-10% cod liver oil/corn oil (2:1 w/v), growth was poor on a lipid free diet (Sheen and D'Abramo, 1991). In juvenile *Penaeus japonicus*, a diet containing 6% lipid (pollack liver oil and soybean oil in a 1:1 ratio) was optimal while 8% lipid from short-neck clam in a casein-based diet produced a positive response compared to purified diets (Kanazawa et al., 1977). In the present study, like in published reports, juvenile *M. rosenbergii* grew well when fed the diet containing 12% total lipid. Estimation of lipid requirements for prawns and shrimps is complicated by the fact that growth is affected by the fatty acid

composition of the diet, its protein:lipid and protein:carbohydrate ratios, and, to some extent, the lipid classes in the feed. However, no study has reported beneficial effects of lipid at 15% or more. The inability of prawn and shrimp to utilize high levels of dietary lipid probably indicates that the lipid level in their natural diet is low. The improved growth of prawns fed diets containing sardine oil is attributed to the presence of HUFA, particularly EPA and DHA.

Survival is equally important for obtaining high production in growout ponds. Survival and growth depend on feed utilization, which is influenced by availability and type of feed, physico-chemical characteristics of the water, and the physiological condition of the cultured animals. In the present study, the highest survival was obtained with the 6.07% diet and the lowest with the control, similar to results in *Tor khudree* fed diets with a low level of marine oil (Bazaz and Keshavanath, 1993).

Specific growth rate (SGR) is an index that assesses diet quality. A higher SGR indicates better utilization and efficient feed conversion. Growth of *M. rosenbergii* can be differential (heterogeneous individual growth) in a normal bi-sex population in growout farming (Murthy, 1998). However, differential growth was not prominent in the present study, probably due to the provision of shelters in the tanks. Feed conversion ratio (FCR) is also a measure of feed utilization. Factors such as water temperature, size of the animal, stocking rate, feed quality, and availability of natural food influence feed conversion, however, there were no significant differences in FCR among treatments in the present study. FCR and protein efficiency ratios (PER) were satisfactory and comparable to earlier findings (Naik and Murthy, 1999; Srinivasa et al., 2003).

RNA:DNA ratios serve as an index of growth per cell with higher ratios indicating growth enhancement. This ratio is a sensitive index of nutrition, where low values are obtained in response to starvation and high values are indicative of good nutrition and rapid growth. In the present study, the highest RNA:DNA was obtained in prawns fed the 14% lipid diet but this ratio did not significantly differ from that of the 12% treatment where prawn growth was highest. Probably, an increase in protein synthesis resulted in higher growth (Srinivasa et al., 2003).

The relative activity of digestive enzymes may correlate with the nature and composition of the consumed food. Variation in the activity of the three enzymes in the present study could have been due to the different levels of sardine oil. Protein accretion was higher in the digestive tract than in the midgut gland. The highest protease activity (total and specific activity) was obtained in the 12% treatment, probably due to the higher levels of essential fatty acids. Proteolytic activities of prawns fed diets containing fishmeal and silage did not statistically differ (Ali et al., 2000). Amylase is one of the most important carbohydrases. Amylase activity was generally high, but there was significant variation among diets. Feeding habits and food type influence amylase activity (Phadate, 1987). Amylase activity (total and specific activity) was significantly higher in the digestive tract and midgut gland of *M. rosenbergii* fed diets containing sardine oil than those fed the control, as in *M. rosenbergii* fed diets containing probiotics (Ali et al., 2000; Srinivasa et al., 2003). In the present study, lipase activity was higher in the hepatopancreas than in the digestive tract. However, in carp fingerlings, lipase activity was higher in the midgut gland (Srinivasa et al., 2003). The protein source has a greater effect on enzyme activity (Lee et al., 1984). While deterioration of water quality is usually associated with accumulation and decomposition of organic matter, the water quality in the present study was within the suitable range for prawn culture (Naik and Murthy, 1999; Naik et al., 2000).

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