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The Effect of Dietary Lipid Levels on the Nutrition and Growth of Juveniles of *Macrobrachium rosenbergii* (de Man)

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The effect of dietary lipid levels on the nutrition and biochemical composition of the giant freshwater prawn *Macrobrachium rosenbergii* (de Man) was studied by incorporating graded levels of cod liver oil in semi-purified diets to provide 2,4,6,8,10,12, and 15 % lipid level. A diet devoid of lipid content was used as control diet. Prawns with 63±10 mg initial body weight were used for the study. Maximum weight gain, better feed conversion and higher protein efficiency ratio were recorded in *M. rosenbergii* juveniles fed with diet containing 6 and 8% dietary lipid level. After 42 days, significantly higher ($p< 0.05$) weight gain was recorded in 4 to 10% dietary levels of lipid than control and 2% lipid diet. However, there exist no statistically significant difference in weight gain, specific growth rate, FCR and protein efficiency ratio among 4 to 10% dietary treatments. The broken line model suggests a requirement of 4.4% lipid level in the diets of *M. rosenbergii* juveniles. Moreover, the results of the present study bring out the negative impact of the over supplementation of lipid (12 and 15%) in the diets of *M. rosenbergii.* The dietary lipid did not affect the survival of prawns and it ranged from 66.11 to 91.11%, however, an addition of 4% dietary lipid increased the survival of prawn to 82.22%. The RNA, DNA and nucleic acid ratios of the muscle tissue of prawns fed with 6 and 8% dietary lipid level were significantly ($p< 0.05$) higher than that of control diet, 2 and 4% dietary lipid levels. A positive correlation was recorded between the dietary and carcass lipid level, however an inverse relationship was noticed between carcass lipid and moisture content. The results of study revealed that dietary lipid had an important role on the nutrition and biochemical composition of juveniles of *M.rosenbergii.*

Key words : *Macrobrachium rosenbergii*, dietary lipid level, lipid requirement.

Lipids are indispensable nutrients for the growth of shrimps and prawns (Kanazawa, 1985) since they form a major source of metabolic energy in prawn and fish. Being highly digestible, lipids have greater sparing action than dietary carbohydrates for protein (Ellis &Reigh, 1991) and they undertake a definite role in the feed utilization also. The levels of lipid used in a diet also vary considerably as per convenience and availability apart from considering the dietary requirements of the target animal. However, information is scanty with regard to the lipid requirements of fresh water prawn *M.rosenbergii* (New, 1976). Adequate knowledge on the nutritional requirements of *M. rosenbergii* is found inevitable for the development of a cost effective feed that can be formulated with some flexibility in the choice of ingredients. Nutritional studies conducted earlier in other crustaceans indicated that the optimal lipid range varies from 5 to 8% of the diet and also found that higher levels of dietary lipid resulted in lower growth rate (Forster & Beard, 1973; Kanawaza *et al.*, 1977; Deshimaru *et al.*, 1979; Davis & Robinson, 1986). Sheen & D'Abramo (1991) reported in *M. rosenbergii* that a dietary lipid level ranging from 2 to 10% under a wide range

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of dietary lipid carbohydrate ratio was found to be satisfactory.

The present study was undertaken to assess the quantitative lipid requirement of *M. rosenbergii* using cod liver oil as dietary lipid source on the growth, survival, feed consumption ratio, protein efficiency ratio and carcass composition of juveniles of *M. rosenbergii*.

Materials and Methods

Iso-nitrogenous and iso-energetic diets were formulated for both the experiments following the semi-purified feed formula (Table 1) in which fat free casein was used as the major protein source. The lipid levels ranged from 0 - 15% of dry weight of the diets. Cod liver oil was used as lipid source. Ingredients were procured from authorised dealers while Cod Liver oil was purchased from Universal Medicare Limited, Sion-Trombay Road, Deonar, Mumbai-400 088.

The feed ingredients were weighed and mechanically homogenised. Butylated hydroxy toluene (BHT) at 0.005 g/100g of dry diet was first mixed with lipid source before adding it in to the dry mixture. Warm water (75 –80° C) was added and mixed thoroughly by hand until a consistency for extrusion was achieved. Diets were hand extruded through a 2mm diameter die. The spaghetti- like strands were steam cooked for 5 minutes and then dried in an electric oven till 8 – 10 % moisture content was obtained. Dried feed strands were broken in to 3 mm pellets and stored in air tight plastic containers at – 4° C. Crude protein, crude lipid, crude fibre, ash and moisture were determined following standard procedures of AOAC (1990) and nitrogen free extract (NFE) was estimated by difference. Digestible dietary energy values were estimated using standard physiological fuel values of 4 kcal/g for protein and carbohydrate and 9 kcal/g for lipid (Maynard *et al.*, 1979).

The feeding experiment was conducted following the randomized block design in order to eliminate the influence of position and place of the tanks among different treatments. For each set of treatment, triplicates were maintained. *M. rosenbergii* juveniles reared in Hatchery Complex of School of Industrial Fisheries with an average weight of 0.063±0.01g were selected and stocked in 100 lit FRP tanks at a stocking density of 15 prawns/tank. In all the experimental groups the animals were fed *ad libitum* for the first week of the experiment at four times a day at 8 AM, 11 AM, 8 PM and at 11 PM to avoid feed wastage and deterioration of water quality. From second week onwards, the pre weighed experimental diets (approximately 15% of the body weight) were placed in petri dishes and unconsumed feed was siphoned out and washed gently with distilled water and filtered through a pre weighed filter paper and dried to constant weight in an electric oven at 60 ° C. Dechlorinated tap water was used as the rearing medium and the level of the water in all experimental tanks were maintained same and 50 % water was exchanged daily at 10 AM. A sponge mope was used to scrub the sides of the tanks on alternate days for any algae or bacterial growth. Water temperature and pH were measured using a mercuric thermometer and pH meter respectively. Dissolved oxygen and ammonia were determined following the standard procedures of APHA (1995).

The initial and final total length of the experimental animal was measured using a vernier caliper from the tip of the rostrum to the tip of the telson. The initial and final live weight of the animals were measured to the nearest 0.0001 g by weighing the individual group of animals in each treatment after removing the water on their body with tissue paper. The growth of the animal in length and live weight was measured with the help of the following standard formulae:

$$\text{Growth} = \frac{(\text{Final measurement} - \text{Initial measurement})}{\text{Initial measurement}} \times 100$$

Specific Growth Rate (SGR) =

$$\frac{\text{(log final weight-log initial weight)}}{\text{Days of experiment}} \times 100$$

Feed Conversion Ratio (FCR) =

$$\frac{\text{Feed consumed (dry weight)}}{\text{Live weight gain (wet weight)}}$$

Protein Efficiency Ratio (PER) =

$$\frac{\text{Live weight gain}}{\text{Protein consumed in dry weight}}$$

Survival % =

$$100 - \frac{\text{(Initial No of prawn- Final No. of prawn)}}{\text{Initial No. of Prawn}} \times 100$$

Approximately 50 prawns were collected at the beginning of the experiment and were stored at –10 °C for the determination of whole body proximate composition from carcass. The feeding experiment was carried out for a period of 42 days and after the termination of experiment all the prawns were weighed, collected and pooled according to the experimental treatments and then sampled for whole body proximate composition analysis and lipase enzyme analysis. Carcass composition analysis for moisture, crude protein, crude lipid and ash were carried out following the standard methodology of AOAC (1990). The nucleic acid content of the muscle tissue of the prawns was estimated following the method of Schneider (1957). The hepatopancreas of the prawn was dissected out at 4 °C and crude enzyme extracts were prepared and then estimated for the total and specific lipase enzyme activity following the standard procedure of Bier (1955).

All the data (except carcass composition analysis) were subjected to analysis of variance (ANOVA) and Duncan's multiple range test to determine the difference between the treatment means (SPSS 7.5 package for WINDOWS). Results were treated for its statistical significance at 0.05 probability level.

Results and Discussion

Water quality parameters like water temperature, pH, dissolved oxygen and ammonia ranged from 27.5 to 29.0°C, 7.3 to 8.2, 5.38 to 6.78 mg/l and <0.2 ppm respectively through out the experimental period. Proximate composition, digestible energy and lipid to carbohydrate ratio of the experimental diets containing different dietary lipid are given in Table 2. The results of the present study emphasize the essentiality of lipid in the diet and also unravel the effect of different levels of lipid on weight gain, feed conversion ratio and protein efficiency ratio in the juveniles of *M. rosenbergii*. The initial and final length, weight and percentage gain in live body length and weight the specific growth rate, feed conversion ratio and survival of *M. rosenbergii* fed with different dietary lipid levels are shown in Table 3. The analysis of variance of the weight gain of prawns fed with varying dietary lipid level showed significant variation ($p<0.05$). Significant weight increase was encountered in prawns fed with diet L8 followed by L6 and L4 when compared to L2 and L0.

No statistically significant difference ($p<0.05$) was observed in the specific growth rate among those fed with L4, L6, L8 and L10 diets. Relatively lower SGR value was obtained in L0, which did not vary significantly from the values registered in respect of L2 and L15. Growth of *M. rosenbergii* juveniles fed with lipid free diet was very poor, however, it was improved significantly by the inclusion of lipids in diets at a level of 4%. Significant increase in weight gain was achieved upon further increase in the level of dietary lipid, even up to 12%. Results of the present study showed that the minimum level of dietary lipid required for optimal growth of *M. rosenbergii* is 4%. Sheen and D'Abramo (1991) while feeding juveniles of *M. rosenbergii* with different dietary levels of cod liver and corn oil mixture, concluded that a dietary lipid level

Table 1. Percentage of ingredients in experimental diets with varying dietary lipid levels

Ingredients	Experimental diets L0	L2	L4	L6	L8	L10	L12	L15
Casein	30	30	30	30	30	30	30	30
Starch	38	35	32	30	28	26	24	20
Cod liver oil	0	2	4	6	8	10	12	15
Aminoacid mix[a]	3	3	3	3	3	3	3	3
Dextrin	5	5	5	5	5	5	5	5
Gelatin	5	5	5	5	5	5	5	5
Sodium citrate	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Mineral & Vitamin mix [b]	2	2	2	2	2	2	2	2
Choline chloride	1	1	1	1	1	1	1	1
Glucosamine HCl	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Vitamin C	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Cholesterol	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Carboxy methyl cellulose	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Alpha cellulose	12.23	13.23	14.23	14.23	14.23	14.23	14.23	15.23

[a] Amino acid mix - 1:1 ratio of Glycine and Betaine
[b] Mineral and vitamin mix ($mg.g^{-1}$ mineral and vitamin mix)
Vitamin A, 625IU; Vitamin D3, 6.25IU; Vitamin E, 0.25 mg; niacinamide, 22.5 mg; thiamin mononitrate, 30mg; riboflavin, 30mg; Folic acid, 50mg; Biotin, 10mg; pryidoxin HCl,9mg; D pantothenate, 37.5 mg; Cyanocobalamine, 0.45 mg; Ca, 280mg P, 120mg; Cu 0.2 mg; I, 1mg; Fe, 6mg; Mn, 1.2mg; Se, 0.01mg; Zn, 2 mg

ranging from 2-10% gave satisfactory results and this finding is almost comparable with the present results. However, in the present study, maximum growth rate was recorded when dietary lipid level was maintained at 8% followed by 6%, however there exist no statistically significant difference between them and this observation was in full accordance with that of Sheen and D'Abramo (1991) who recorded higher percentage increase in body weight at 6% followed by 8% dietary lipid level. However, in the present study the broken line model of the specific growth rate has a break point at 4.4% dietary lipid level (Fig.1) and this would suggests the exact lipid requirement for *M.rosenbergii* juveniles for normal growth.

It appears that inclusion of more than 4% lipid in the diet of *M. rosenbergii* resulted in non significant improvement in growth performance of prawns while higher dietary lipid level (15%) showed a synergetic effect on growth performance. This finding shows strong agreement with that of Kanazawa *et al.* (1977) who used powdered pollack residual oil as a lipid source for *Penaeus japonicus* and found that the body weight was lower when the dietary level was increased to 16%. When the oil content of a diet for *Palaemon serratus* was increased from 7.5 to 15%, the growth rate showed a significant reduction (Forster & Beard, 1973).

The feed conversion ratio worked out in the present study showed significant variations ($P<0.05$) as lower FCR values were recorded for *M. rosenbergii* juveniles fed with diet L8 and L6 when compared to diet L0 and L2. The differences in FCR among diets L4 to L15 were not statistically significant ($P>0.05$). The results of ANOVA did not found show any variation in protein efficiency ratio among the treatments ($p>0.05$). However, Duncan's test showed significantly higher PER values in diet L8 and L6 in contrast to diets L0 and L2. In higher dietary lipid levels (L12 and L15), the protein efficiency ratio was not found better than that of L4, L6 and L8. Low growth and

Table 2. Proximate compositon, energy content and protein to energy ratio of diet containing different dietary lipid levels

Experimental Diets	L0	L2	L4	L6	L8	L10	L12	L15
Moisture (%)	8.86	9.45	8.69	9.32	8.79	8.86	9.45	9.15
Crude protein (%)	35.24	35.10	35.16	35.20	35.18	34.98	35.23	34.93
Crude lipid (%)	0.12	2.10	4.17	6.12	8.10	10.20	12.05	15.24
Ash (%)	3.58	3.98	4.23	4.97	4.68	4.69	3.98	4.29
Crude fiber (%)	10.35	11.95	12.75	12.35	12.86	12.56	12.45	13.68
NFE (%) [1]	41.85	37.42	35.00	32.04	30.39	28.71	26.84	22.71
Energy (kcal/100g) [2]	309.44	308.98	318.17	324.04	335.18	346.56	356.73	367.72

[1] By Difference

[2] Calculated apparent digestible energy= [4(%protein)+9(% lipid)+4(%carbohydrate)]

Table 3. Weight gain, percentage increase in weight SGR, FCR, PER, survival rate, RNA, DNA, muscale tissue and lipase enzyme activity of hepatopancrease RNA, DNA and nucleic acids ratio of muscle tissue and lipase enzyme activity of hepatopancrease of Macrobrachium rosenbergii juveniles fed with different dietary lipid levels.Mean value with different superscript vary significantly ($p<0.05$)

	L0	L2	L4	L6	L8	L10	L12	L15
Initial weight (g)	0.063 ± 0.004	0.057 ± 0.001	0.059 ± 0.005	0.063 ± 0.005	0.060 ± 0.005	0.059 ± 0.002	0.061 ± 0.007	0.063 ± 0.008
Final weight (g)	0.141 ± 0.027	0.151 ± 0.032	0.210 ± 0.024	0.242 ± 0.033	0.246 ± 0.044	0.231 ± 0.069	0.204 ± 0.042	0.174 ± 0.02
Weight gain (g)	0.078 ± 0.03^{a}	0.094 ± 0.033^{ab}	0.161 ± 0.022^{bcd}	0.179 ± 0.033^{cd}	0.186 ± 0.039^{d}	0.172 ± 0.067^{cd}	0.143 ± 0.04^{bcd}	0.112 ± 0.013^{ab}
% Weight gain	125.04 ± 54.74^{a}	164.96 ± 60.9^{ab}	271.16 ± 35.67^{cd}	286.63 ± 53.14^{d}	310.07 ± 39.86^{d}	289.00 ± 105.5^{d}	232.06 ± 38.93^{bcd}	178.43 ± 11.09^{ab}
SGR	1.89 ± 0.55^{a}	2.27 ± 0.59^{a}	3.11 ± 0.24^{cd}	3.21 ± 0.32^{cd}	3.35 ± 0.23^{d}	3.17 ± 0.70^{cd}	2.85 ± 0.27^{abc}	2.44 ± 0.01^{abc}
FCR	3.21 ± 0.06^{b}	3.12 ± 0.02^{b}	2.95 ± 0.18^{ab}	2.82 ± 0.08^{a}	2.80 ± 0.16^{a}	3.05 ± 0.09^{ab}	3.00 ± 0.19^{ab}	2.98 ± 0.23^{ab}
PER	0.66 ± 0.42^{a}	0.67 ± 0.21^{a}	0.94 ± 0.07^{ab}	1.13 ± 0.26^{b}	1.15 ± 0.21^{b}	1.01 ± 0.18^{ab}	0.87 ± 0.09^{ab}	0.84 ± 0.06^{ab}
Survival %	66.67 ± 6.67^{a}	68.89 ± 3.85^{a}	82.22 ± 10.18^{a}	86.67 ± 17.64^{a}	86.67 ± 13.33^{a}	91.11 ± 10.18^{a}	84.44 ± 21.43^{a}	75.56 ± 7.70^{a}
RNA (mg/gm wt tissue)	14.49 ± 2.23^{a}	15.91 ± 0.47^{a}	17.09 ± 0.34^{bc}	18.42 ± 0.10^{bc}	19.58 ± 0.93^{d}	$18.61\ 1.89^{cd}$	16.78 ± 1.66^{abc}	16.06 ± 0.78^{bc}
DNA (mg/gm wt tissue)	9.42 ± 0.33^{a}	9.49 ± 0.35^{a}	9.73 ± 0.67^{a}	10.33 ± 0.18^{b}	10.31 ± 0.26^{b}	10.17 ± 0.34^{ab}	10.02 ± 0.44^{ab}	9.93 ± 0.69^{ab}
RNA/DNA	1.54 ± 0.26^{a}	1.68 ± 0.02^{abc}	1.76 ± 0.11^{bcd}	1.78 ± 0.11^{d}	1.90 ± 0.10^{d}	1.83 ± 0.21^{d}	1.68 ± 0.24^{abc}	1.62 ± 0.14^{ab}
Total Lipase Activity*	0.31 ± 0.020^{a}	0.30 ± 0.03^{a}	0.32 ± 0.02^{b}	0.44 ± 0.07^{c}	0.42 ± 0.01^{c}	0.43 ± 0.03^{c}	0.44 ± 0.01^{c}	0.43 ± 0.01^{c}
Specific Lipase activity**	0.03 ± 0.001^{a}	0.32 ± 0.001^{b}	0.03 ± 0.002^{b}	0.04 ± 0.001^{c}	0.05 ± 0.001^{de}	0.05 ± 0.001^{e}	0.05 ± 0.01^{f}	0.04 ± 0.01^{d}

* 0.02 N NaOH required/min per g tissue.

** 0.02N NaOH required/min/mg protein.

reduced protein efficiency ratio in prawns fed with lower lipid levels (0 and 2%) can be attributed to a condition in which the feed and the protein are poorly utilised. This observation shows full agreement with that of Chandge and Raj (1997). From the results of the present study, it can be inferred that lipid at adequate levels can significantly spare protein for growth as already been established in fish by Watanabe (1982).

The dietary lipid level did not influence the survival rate of juvenile prawn and it ranged from 66.67 to 91.11%. However, the survival was low in-group of *M. rosenbergii* fed with lipid free diet L0. Addition of 4% dietary lipid level improved the survival to 82.22%. Diet containing 4,6,8,10 and 12% of dietary lipid showed relatively high survival, on the contrary, comparatively lower survival was encountered in diet with 15% lipid level. Based on the present study it can be inferred that the dietary inclusion level of lipid alone did not affect the survival. Similar results were reported in *M. rosenbergii* fed with different levels of cod liver oil and corn

Table 4. Carcass composition analysis of juvenile *Macrobrachium rosenbergii* fed with diets containing varying dietary lipid levels

	Initial	L0	L2	L4	L6	L8	L10	L12	L15
Moisture content (%)	72.32	74.79	74.82	73.85	74.68	74.32	73.1	72.27	71.34
Crude protein (%) *	65.23	65.45	65.84	68.56	70.56	70.94	70.98	69.54	68.95
Crude Lipid (%) *	5.72	5.23	5.81	5.98	6.10	6.07	6.87	7.22	7.72
Ash (%) *	13.96	14.98	14.25	13.86	14.71	14.32	14.05	13.96	14.47

* Dry weight basis

oil mixtures (Sheen & D'Abramo, 1991). In *Penaeus indicus* the survival was affected significantly by dietary lipid level and this was resolved by the an addition of 3% dietary lipid (Chandge & Raj, 1997). In the present study also, supplementation of 4% dietary lipid level had resulted an improvement of the survival rate from 66.67% in fat free diet (L0) to 82.22% when lipid level was maintained at 4%.

RNA, DNA and RNA/DNA ratio of the muscle tissue and total and specific lipase activity of hepatopancreas of *M. rosenbergii* fed with different dietary lipid sources are shown in Table 3. The RNA content of muscle tissue of *M. rosenbergii* juveniles showed significant variation ($p<0.05$) and the highest RNA content was estimated in prawns fed with diet containing 8%. The lowest RNA content was recorded in fat free diet and diet with 2% and 15% lipid level. The nucleic acid ratios in the muscle tissue of *M. rosenbergii* were also found significantly lower in 0, 2, 4 and 15% dietary lipid level than 6, 8 and 10%. The total and specific lipase activity of hepatopancreas of *M. rosenbergii* showed significant difference among different dietary treatments. *M.rosenbergii* juveniles fed with diet containing 0, 2 and 4% lipid levels showed significantly lower total and specific lipase activity when compared to other dietary treatments and this can be attributed to sub-optimal level of dietary lipid levels.

The results of carcass composition analysis of *M. rosenbergii* fed with different inclusion levels of dietary lipid are presented in Table 4. An increase in the body protein content was recorded in prawns fed with diets having up to 10% dietary lipid, although the carcass lipid content increased with increase in dietary level of lipid. A negative correlation (r^2 = 0.90 and Y= - 1.5x + 83.2) was observed between the percentage of moisture and lipid in the body (Fig 2), whereas the correlation was positive (r = 0.94 and Y= 0.1557x + 5.266) between the dietary lipid and the carcass lipid level (Fig. 3). A positive relationship between the dietary lipid and the carcass lipid observed in the present study agrees with Sheen & D'Abramo (1991) and Hilton *et al.* (1983). An inverse relationship noticed between carcass fat and carcass moisture in the present study corroborates fully with that of Chandge & Raj (1997) in *P. indicus*.

A steady increase in growth performance and protein content of the prawn with the increase in dietary level of lipid from 0 to 8% may be attributed to the protein sparing action of these dietary lipids. The results of the present study showed that the dietary lipid level above 8% had no beneficial effect on the food and protein utilisation of prawns. Protein utilisation was found to be better when enough fat and carbohydrate were provided in their diet. Clifford & Brick (1978) made similar observations in *M. rosenbergii*. While Chandge & Raj (1997) clearly demonstrated the protein sparing effect of lipid in Indian white shrimp *Peneaus indicus*

In conclusion, higher weight gain, better feed conversion and higher protein efficiency ratio were recorded in *M. rosenbergii* juveniles fed with diet containing 6 and 8% dietary lipid level. Further increase in dietary lipid had no corresponding effect in improving growth performance. Growth performance showed a perceptible decline at high concentrations (15%) of dietary lipid levels. and was improved significantly by the inclusion of lipids in diets at a level of 4% and no improvement in weight gain was noticed with further increase in dietary lipid level even up to 12%. The broken line model suggested a lipid requirement of 4.4% in the diets of *M.rosenbergii* of normal growth.

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