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Effect of papain supplemented diet on growth and survival of post -larvae of *Macrobrachium rosenbergii*

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Abstract
The study was conducted to evaluate the effect of papain supplemented diet on growth and survival of post-larvae of *Macrobrachium rosenbergii*. The enzyme papain (LOBA CHEMIE PVT LTD., Bombay, INDIA) was supplemented at the rate of 0.1, 0.2, 0.3 and 0.4% in control diet containing 35% protein and served as experimental diet viz. T_1, T_2, T_3 and T_4. Diet without enzyme supplementation served as control diet viz. T_0. The feeding experiment was conducted for 42 days rearing period following Complete Randomized Design (CRD) with four replicates for each treatment.
Maximum length gain (18.36%), weight gain (81.15%) and Specific Growth Rate (1.41%) were observed in post-larvae fed with T_1 diet (0.1%) and it was found significantly different ($P<0.05$) from T_0 diet but not significantly different ($P>0.05$) from T_2, T_3 and T_4 diets. The maximum survival (59.33%) was observed for post-larvae fed with T_1 diet and it was not significantly different ($P>0.05$) from the control and other experimental diets.
The results of this feeding trial indicated that the 0.1% supplementation of papain to the diet resulted in better growth and feed utilization of post-larvae of *M. rosenbergii*.

Keywords: Growth, survival, post-larvae of M. rosenbergii, papain

1. Introduction
Among the aqua culturist the prawn farming gets more popularity in order to meet the growing demand for delicious and nutritious prawn all over the world. In semi-intensive and intensive prawn farming feed is the major constituent of variable cost comprising up to 60% of the total production cost [1]. Therefore, it is necessary to develop nutritionally adequate and cost effective feed for prawn farming. Efficient utilization of feed and maximum utilization of all nutrients of the diet are the areas of prime concern in feed management for the prawn farming.
Digestion efficiency of the cultured species can be increased by supplementing the enzymes to the feed [6]. However, there is very less scientific study on the use of enzymes in the diet of post-larvae of *M. rosenbergii*. Hence the present study was designed to study the effect of papain supplemented diet on growth and survival of post-larvae of *M. rosenbergii.*

2. Materials and Methods
2.1 Test animal
Post-larvae of *M. rosenbergii* were obtained from the freshwater prawn hatchery of Marine Biological Research Station, Ratnagiri and maintained in 500 L plastic pool. They were acclimatized for one week to the laboratory conditions and were fed three times per day with control diet (T_0) at the rate of 10 % body weight [13]. Faeces and remaining feed were removed from the culture tank by siphoning out 25 % of water daily, which was replaced back with new freshwater. Aeration was provided throughout acclimatization period to avoid stress.

2.2 Diet preparation
2.2.1 Control diet
Control diet used for experimental purpose was prepared by using fishmeal and groundnut oil cake as a dietary protein source while rice bran and wheat flour as basal sources. Diet contained 35% protein on the basis of nutritional information available for *M. rosenbergii* [3, 5, 12].

The required quantity of finely powdered and sieved ingredients were precisely weighed and mixed thoroughly in a domestic mixer for one minute. Afterwards 350 ml water was added to it and further it was mixed in domestic mixer to form slurry. The slurry was steamed for 15 minutes and cooled to room temperature for 20 minutes. Afterward the cooled slurry was spread on a polythene sheet in 2 to 3 mm thick layer with the help of soft brush (New Wilson, 100 mm size). Then it was dried for 3 hours to from flacks. After sun drying, the flaks were broken into small pieces of 2 to 3 mm size and stored in plastic container.

2.2.2 Experimental diet

The enzyme papain (LOBA CHEMIE PVT LTD, Bombay) was supplemented at the rate of 0.1, 0.2, 0.3 and 0.4 % in control diet and served as experimental diet *viz.* T_1, T_2, T_3 and T_4. The enzyme was dissolved in 50 ml water and mixed thoroughly in a cooled slurry of diet in domestic mixer. The method of preparation of the experimental diets was similar to that of the control diet.

2.3 Ingredients and proximate composition

Ingredients and proximate composition of control and test diets are given in table 1. The diets were analyzed for moisture content [2] and nitrogen content was determined by Micro-kjeldhal method [2]. Protein was calculated by multiplying nitrogen content by a constant 6.25. Crude fat content was analyzed by Soxhlet extraction with petroleum ether [2]. Ash content was estimated after incineration of sample at 550 ^{0}C in a muffle furnace for 5 hours [2]. The carbohydrate was computed by reminder method [16]. Gross energy in each of the diets was calculated using conversion factors carbohydrate, 4.1; fat, 9.5; and protein, 5.65 [10].

Table 1: Ingredients (%) and proximate composition (% dry weight basis) of the diets fed to post-larvae of *M. rosenbergii* for 42 days.

Ingredients and proximate composition	Test diet				
	T_0	T_1	T_2	T_3	T_4
Ingredients (%)					
Fish meal	29.21	29.21	29.21	29.21	29.21
Groundnut oil cake	29.21	29.21	29.21	29.21	29.21
Wheat flour	20.79	20.79	20.79	20.79	20.79
Rice bran	20.79	20.79	20.79	20.79	20.79
Papain	--	0.1	0.2	0.3	0.4
Proximate composition (%)					
Moisture*				10.23	
Crude protein*	10.89	9.93	10.90	34.00	9.65
Crude lipid*	34.00	35.00	35.00	5.13	36.00
Ash*	5.20	5.59	5.00	9.50	4.87
Carbohydrate*	10.50	9.44	10.10	41.14	10.25
Gross energy (kcal/100 g)	39.41	40.04	39.00	413.62	39.23
Protein / energy ratio (mg	407.02	419.02	409.05	82.20	414.43
protein / kcal)	83.53	83.53	85.56		86.87

*Mean of two estimations.

2.4 Experimental Procedure

Feeding experiment was conducted for 42 days following Completely Randomized Design with four replicates for each treatment. Plastic circular tubs of 30 L capacity with 0.18-m2 base were filled with 20 L of freshwater. Post-larvae of *M. rosenbergii* (2.016±0.07, 2.017±0.10, 2.017±0.06, 2.017±0.07 and 2.025±0.06 cm length and 0.061±0.008, 0.064±0.010, 0.064±0.007, 0.063±0.008 and 0.084±0.007 g weight) were randomly stocked in experimental tubs at the rate of 30 post-larvae per tubs for T_0, T_1, T_2, T_3 and T_4 respectively. Post-larvae were fed at the rate of 10 % body weight and the ration was given three times a day at 11.00, 15.00 and 19.00 hrs. Due to nocturnal feeding behavior evening ration was more as compared to other two rations. Small pieces of Mangalore tiles were kept in each tub as a shelter for molted post-larvae. Uneaten feed and feaces was removed daily at 10.00 hrs. along with 25% of siphoned out water from each tub while entire water was completely changed once a week. Each tub was aerated with one diffuser type air stone.

At the beginning of the experiment individual wet weight after bolting the excess moisture with the help of blotting paper and length were recorded to nearest 0.01 mg and 1 mm respectively. Biomass of each tub and individuals of each tub counted weekly. On termination of experiment length and weight of all the animals were recorded and the following observations were calculated as per Shinde [12].

$$\text{Weight gain (\%)} = \frac{\text{(Final weight – Initial weight)}}{\text{Initial weight}} \times 100$$

$$\text{Length gain (\%)} = \frac{\text{(Final length – Initial length)}}{\text{Initial length}} \times 100$$

$$\text{SGR (\%)} = \frac{\text{(In final weight – In initial weight)}}{\text{In initial weight}} \times 100$$

$$\text{Survival (\%)} = \frac{\text{Initial count – Final count}}{\text{Initial count}} \times 100$$

2.5 Statistical Analysis
Data obtained in feeding experiments were analyzed by one-way ANOVA. Significant difference was indicated at 0.05 level, the Newman Keul's multiple comparison test was used. (14, 17).

3. Result and discussion
Length gain (%), weight gain (%), specific growth rate (%) and survival of post-larvae fed with control and experimental diets for 42 days period is given in Table 2. Maximum length gain (18.36%), weight gain (18.45%) and specific growth rate (1.41%) were recorded in post-larvae fed with T_1 diet.

One-way ANOVA showed significant difference ($P<0.05$) between the diets for length gain, weight gain and specific growth rate. Newman Kuel's multiple range test reveled that the post-larvae fed with T_1 diet was not significantly different ($P>0.05$) from other experimental diets, but significantly different ($P<0.05$) from the post-larvae fed with the control diet. This study showed that the low level incorporation of papain in practical diet of *M. rosenbergii* resulted in better growth of post-larvae of *M. rosenbergii* which was cost effective.

There was no report on the use of papain in the diet for post-larvae of *M. rosenbergii* but some workers studied the effect of photolytic enzymes on other species. Maugle *et al.* [11] reported the dietary supplements of bovine trypsin resulted in better growth for *P. japonicus.* It may be due to the activation of endogenous protease zymogen(s) in the digestive system by exogenous bovine trypsin resulted in increased digestive capacity of hepatopancreas. Divakaran and Velasco [9] reported contradictory results in shrimp, *Laptopeanes vannamei* fed with proteolytic enzymes.

Dabrowski and Glogowski [8] observed increased proteolytic activity in common carp fed with diet containing bovine trypsin and stated that there was a new approach to use the proteolytic enzymes to fish diets for better growth. Carter *et al.* [4] reported better growth in *Salmo salar* by supplementing proteolytic enzymes to the diets. Similar results also observed for common carp fed with synthetic feed mixed with 3% papain, artificial feed mixed with 5% papain and synthetic feed mixed with 10% papaya leaf by Tagare [15]. He reported that better growth in papain supplemented diet was due to the increase in protein digestion in common carp.

Thus, from above literature, it is easy to say that the proteolytic enzymes play a role in protein digestion as reported by Maugle *et al*. [11] in shrimp and Tagare [15] in fish. During present study diet supplemented with 0.1 % of papain resulted in better growth of post-larvae of *M. rosenbergii.* This may be due to the increase in digestion capacity of post-larvae because of the ready availability of papain along with feed.

In the present study maximum survival (57.17%) was observed in post-larvae fed with T_1 diet in 42 days rearing period. But there was no significant difference ($P>0.05$) for survival of post-larvae fed with different diets. Dabrowski and Glogowski [7] reported that proteolytic enzymes exogenously added to fish feed do not show any effect on survival of fish. Similar results also made by Tagare [15] in common carp fed with diet containing papain and crude papain in form of papaya leaf.

Table 2: Length, weight, specific growth rate and survival of post-larvae of *M. rosenbergii* fed with diets during experimental period.

Growth parameter	Test diet				
	T_0	T_1	T_2	T_3	T_4
Length gain (%)	10.13±0.77 ^{a}	18.36±0.66 ^{b}	17.43±0.72 ^{b}	16.89±0.63 ^{b}	15.52±0.78 ^{b}
Weight gain (%)	45.47±3.07 ^{a}	81.15±5.59 ^{b}	71.59±2.91 ^{b}	69.22±3.03 ^{b}	68.38±3.01 ^{b}
Specific growth rate (%)	0.89±0.02 ^{a}	1.41±0.03 ^{b}	1.29±0.01 ^{b}	1.25±0.01 ^{b}	1.25±0.01 ^{b}
Survival (%)	52.42±7.79 ^{a}	59.17±8.97 ^{a}	53.33±8.27 ^{a}	58.33±7.76 ^{a}	54.17±10.31 ^{a}

Note: Values are Average ± S. E.; Values within same row with same superscript are not significantly different at 0.05 probability level.

4. Conclusion
The present study concluded that a diet supplemented with 0.1% papain is suitable for the better growth and maximum nutrient utilization from feed for rearing of post-larvae of *M. rosenbergii*.

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