

Probiotic based diets for freshwater prawn *Macrobrachium rosenbergii* (de Man)

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ABSTRACT

Experimental trials were conducted to investigate the impact of incorporation of commercial probiotics viz, “Improval” and “Prosol”, in supplementary diets of *Macrobrachium rosenbergii* post-larvae and juveniles. The experiment was conducted in tanks of 350 l capacity for 105 days (60 days for post-larvae and 45 days for juveniles). For each dietary treatment, triplicates were maintained. Ten experimental diets were formulated with different levels of probiotics. In prawns fed with diet containing 4% Improval and 2-3% of probiotic Prosol, significantly higher ($p < 0.05$) final body weight, net body weight gain and specific growth rate were recorded. In addition, the apparent feed conversion ratio and protein efficiency ratio in probiotic dietary treatments were significantly higher ($p < 0.05$) than for control diet. The results of our study suggests that the addition of probiotics, as growth promoter, in the diet may be useful for profitable culture of *M. rosenbergii*.

Keywords: Growth performance, Improval, *Macrobrachium rosenbergii*, Probiotic, Prosol

Introduction

Aquaculture has emerged as one of the most promising and fastest growing food producing sector around the globe, which provides avenues for high quality protein, income generation, employment and foreign exchange earnings. Recent developments in the aquaculture sector and the increasing demand for fish production resulted in intensification of the aquaculture practices. Intensive culture systems create highly stressful environment for the cultured fish that further suppresses the immune response, leading to outbreak of infectious diseases (Saurabh and Sahoo, 2010). Probiotics are beneficial microorganisms that protect the host from diseases. Fuller (1992) defined probiotics as ‘live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance’. Microbes play very important and crucial role in aquaculture systems, both at hatchery and grow-out levels, as water quality as well as disease incidences are directly affected by microbial activity (Kumar *et al.*, 2006). The range of probiotics examined for use in aquaculture has encompassed both Gram-negative as well as Gram-positive bacteria, yeast and unicellular algae. In particular, probiotics have been reported to be successful with a wide range of invertebrates (Requelme *et al.*, 2000; Lin *et al.*, 2004; Ziaei-Nejad *et al.*, 2006).

In aquaculture, most probiotics are supplied as live supplements in diets, which have the ability to survive passage through the intestinal tract (Fuller, 1992). Several

mechanisms have been suggested, contributing to the beneficial effects of probiotics, including the removal of pathogens by the beneficial population, which is often considered as the most important mechanism (Gatesoupe, 1999; Gomez-Gill *et al.*, 2000). Some studies have attributed the enhancement of animal growth to the nutritional benefits of probiotic bacteria, such as vitamin production, availability of minerals as well as trace elements and production of important digestive enzyme (Holzapfel *et al.*, 1998). The use of lactic acid bacteria (LAB) as probiotics and non-specific immunostimulants (Verschuere *et al.*, 2000) has been proposed, in addition to their effects on improved water quality (Skjermo and Vadstein, 1999) and nutrition (Ronnestad *et al.*, 1999) as a means to increase larval survival and aquaculture output. There has been great interest in the use of LAB as probiotic in aquaculture industry (Gatesoupe, 2002; Lara-Flores, 2003; Planas *et al.*, 2004).

Though, probiotics have been shown to be effective in a wide range of species for growth promotion, enhanced nutrition, immunity and survival rate, very few attempts have been made to investigate the effect of immunostimulants and/or probiotics for growth improvement of freshwater prawn, *Macrobrachium rosenbergii* (Suralikar and Sahu, 2001; Venkat *et al.*, 2004). The present study investigated the effects of supplementing commercially available probiotics viz., Improval and Prosol on growth performance during post-larval and juvenile life stages of *M. rosenbergii*.

Materials and methods

Test animals

Post-larvae of giant freshwater prawn, *M. rosenbergii* were procured from the freshwater prawn hatchery of the Research Centre of the Central Institute of Fisheries Education (CIFE) at Lalhi, Rohtak, Haryana, and were acclimatised in plastic tanks for 30 days. Post-larvae were fed to satiation with commercially available prawn feed and mixed zooplankton. Daily 50% water was removed from the plastic pools, along with uneaten feed and excreta, and replenished with same quantity of freshwater. Continuous aeration was provided in the tanks using an air compressor in order to ensure oxygen saturation.

Experimental diets

In the present study, two experimental trials were conducted side by side with two commercially available probiotic supplements viz., 'Improval' (Cadila Healthcare Limited, Ahmedabad, India) derived from *Lactobacillus sporogenes* and *Saccharomyces cerevisiae* and 'Prosol' (Intvet Products Mfg. Co. Pvt. Ltd., Salem, India) derived from *Lactobacillus acidophilus*, *L. reuteri*, *L. lactis*, *L. fermentum*, *L. casei*, *Bifidobacterium bifidum*,

Streptococcus faecium, *Aspergillus oryzae*, *Torulopsis* and Vitamin-C). The ingredient compositions of experimental diets containing Improval and Prosol are shown in Table 1 and 2, respectively. For each probiotic supplement, five isonitrogenous (35% crude protein) experimental diets were prepared at different levels viz., 0% (control diet, D0), 2% (D1), 4% (D2), 6% (D3) and 8% (D4), with corresponding decrease in the amount of rice bran for Improval treatment (Table 1), and 0% (control diet, F0), 1% (F1), 2% (F2), 3% (F3) and 4% (F4), with corresponding decrease in the amount of wheat flour for Prosol treatment (Table 2). The ingredients excluding sunflower oil, probiotic supplement and vitamin and mineral mixture were mixed with water and cooked in a pressure cooker for 30 min. After cooking, heat sensitive ingredients were added and the dough was pelletised (0.2 cm dia pellets) in a locally made motorised pelletizer. The resulting pellets were dried at 60 °C for 12 h and broken in to crumbles of 1 mm size. The chemical composition of both the probiotic diets are presented in Table 1 and 2, respectively. The diets were analysed following standard procedures (AOAC, 2000). Gross energy and digestible energy of the diets were calculated, following Brett (1973) and NRC (1993), respectively.

Table 1. Ingredient and chemical composition of the experimental diets supplemented with the probiotic, Improval

Parameter	Diet				
	D0	D1	D2	D3	D4
<i>Ingredient composition (%)</i>					
Fish meal	30	30	30	30	30
Meat cum bone meal	10	10	10	10	10
Soybean meal	30	30	30	30	30
Mustard oilcake	10	10	10	10	10
Rice bran	12	10	8	6	4
Corn starch	4	4	4	4	4
Improval	0	2	4	6	8
Sunflower oil	3	3	3	3	3
Vitamin and mineral premix	1	1	1	1	1
<i>Chemical composition (%)</i>					
Dry matter	89.88	90.1	90.32	90.54	90.76
Moisture	10.12	9.90	9.68	9.46	9.24
Crude protein	35.69	35.43	35.16	34.90	34.64
Lipid	5.62	5.30	4.97	4.65	4.33
Ash	8.82	8.69	8.55	8.42	8.29
Organic matter	81.06	81.41	81.77	82.12	82.47
Carbohydrate	39.74	40.68	41.63	42.57	43.50
Gross energy (kcal 100 g ⁻¹)	417.69	417.05	416.30	415.66	414.98
Digestible energy (kJ g ⁻¹)	19.27	19.52	19.77	20.01	20.26

Number of determinations = 3

Table 2. Ingredient composition and chemical composition of the experimental diets supplemented with the probiotic, Prosol

Parameter	Diets				
	F0	F1	F2	F3	F4
<i>Ingredient composition (%)</i>					
Fish meal	30	30	30	30	30
Meat and bone meal	20	20	20	20	20
Soybean meal	30	30	30	30	30
Wheat flour	10	9	8	7	6
Corn starch	5	5	5	5	5
Prosol	0	1	2	3	4
Sunflower oil	4	4	4	4	4
Vitamin and mineral premix	1	1	1	1	1
<i>Chemical composition (%)</i>					
Dry matter	90.2	90.4	90.5	90.6	90.7
Moisture	9.72	9.59	9.48	9.31	9.29
Crude protein	35.25	35.12	35.00	34.87	34.7
Lipid	4.13	4.12	4.10	4.08	4.07
Total ash	10.26	10.23	10.20	10.17	10.15
Organic matter	80.02	80.17	80.32	80.47	80.6
Carbohydrate	40.64	40.93	41.22	41.51	41.80
Gross energy (kcal g ⁻¹)	404.8	405.1	405.4	405.8	406.1
Digestible energy (kJ g ⁻¹)	19.36	19.44	19.51	19.59	19.67

Number of determinations = 3

Feeding experiment

The present experimental trials with the commercial probiotics, Improval and Prosol were conducted separately during post-larval and juvenile life stages of freshwater prawn. Thirty post-larvae each with an average body weight of 0.38 g and 0.45 g were stocked in 350 l capacity plastic tanks (1.2 x 1.06 x 0.73 m) for the feeding trials with Improval and Prosol respectively. For each treatment, triplicates were maintained. Continuous aeration was given and water exchange was done at the rate of 50% per day. Feeding was done twice daily (0900 and 1600 hrs) at the rate of 10% of the body weight. For juvenile experiment, 5 nos. (@50000 nos. ha⁻¹) of juveniles each with average body weight of 7.15 and 7.26 g, were stocked for feeding trials with Improval and Prosol, respectively. Feeding was done twice daily at the rate of 5% of the body weight.

Water quality and growth parameters

The samples for water quality parameters were taken at weekly intervals and analysed, following standard procedures (APHA, 1989). Fortnightly sampling was done to estimate the average weight and survival of the prawns and also to adjust the feeding rate. At the end of 60 and 45 day feeding trial, the water from each pool was siphoned out and the total number of prawns was counted and individual body weights were recorded. The growth

performance was calculated in terms of specific growth rate (SGR), apparent feed conversion ratio (AFCR) and protein efficiency ratio (PER), as per standard procedures. Feed conversion ratio was considered to be apparent, as no correction was made for uneaten food.

Data analyses

Data were statistically processed for one-way analysis of variance (ANOVA) to find out any significant differences among the experimental groups and the comparison between two treatments was made, using Duncan's multiple range test according to Snedecor and Cochran (1961).

Results and discussion

Water quality

During the experimental trials, all the water quality parameters viz., temperature (27.5-29.2 °C); pH (7.3-7.7); dissolved oxygen (6.1-8.5 mg l⁻¹); free carbon-dioxide (nil) and alkalinity (112-119 mg l⁻¹) in the experimental tanks remained within the acceptable range, as required for freshwater prawn culture (Gupta *et al.*, 2007).

Growth performance of prawns supplemented with probiotic Improval

The growth performance of the freshwater prawn fed with different experimental diets, supplemented with

Improval, revealed that diets incorporated with the probiotic exhibited higher growth at all levels of supplementation, compared to those fed with control diet.

During post-larval stage, the highest ($p<0.05$) performance in terms of average final body weight (16.14 g), net body weight gain (15.76 g) and specific growth rate (7.50 %bw d⁻¹) were recorded in prawns fed with diet containing 4% (D2) Improval (Table 3). In addition, AFCR and PER of prawns fed with diets D2 (1.88 and 1.44, respectively) and D3 (1.84 and 1.43, respectively), supplemented with 4 and 6% of probiotic Improval, were significantly better ($p<0.05$) than those fed with other diets (Table 3). The best survival rate (80%) was observed for the group fed on diet D2. The probiotic Improval also significantly improved the survival of juveniles and the best survival (80%) was observed in D2 (Table 3). The highest significant values ($p<0.05$) of average final body weight (26.64 g), net body weight gain (19.49 g) and specific growth rate (2.63 %bw d⁻¹) were observed in prawns fed diet supplemented with 4% of Improval (D2), whereas prawns fed on control diet recorded the lowest value (Table 3). The AFCR and PER in juveniles fed on diet D2 (1.62 and 1.67, respectively) supplemented with 4% of probiotic were significantly better ($p<0.05$) than the juveniles fed with other experimental diets (Table 3).

Growth performance of prawns supplemented with probiotic Prosol

Growth performance of the freshwater prawn post-larvae and juveniles fed diets supplemented with probiotic Prosol are presented in Table 4. The diets significantly improved the survival of freshwater prawn and the best survival was observed in F3 (76.6%) during post-larval stage and in F2 (80%) during juvenile stage. The highest significant values ($p<0.05$) of average final body weight (13.35 g), net body weight gain (12.9 g) and specific growth rate (6.78 %bw d⁻¹) were noticed in post-larvae fed diets supplemented with 3% of Prosol (F3), whereas prawns fed on control diet showed the lowest value (Table 4). However, during juvenile stage the highest significant values ($p<0.05$) of average final body weight (23.96 g), net body weight gain (16.70 g) and specific growth rate (2.39 %bw d⁻¹) were recorded in juveniles fed diet supplemented with 2% of Prosol (F2). The AFCR and PER in post-larvae fed on diet F3 (2.09 and 1.33, respectively) and juveniles fed on F2 (1.77 and 1.63, respectively) were significantly better ($p<0.05$) than those fed with other experimental diets (Table 4).

The supplementation of the probiotic Improval and Prosol, as growth promoter, significantly improved the

Table 3. Growth performance and feed utilisation of *M. rosenbergii* post-larvae and juveniles fed diets supplemented with the probiotic Improval

Nutritional indices	Diets				
	D0	D1	D2	D3	D4
<i>Post-larvae</i>					
Initial body weight (g)	0.38±0.02 ^a	0.38±0.02 ^a	0.38±0.02 ^a	0.38±0.02 ^a	0.38±0.02 ^a
Final body weight (g)	6.28±2.35 ^d	9.82±3.05 ^c	16.14±3.57 ^a	12.56±2.60 ^b	9.77±2.97 ^c
Net body weight gain (g)	5.9±3.98 ^d	9.44±2.35 ^c	15.76±3.24 ^a	12.18±2.68 ^b	9.39±2.15 ^c
Specific growth rate (%bw d ⁻¹)	5.61±0.26 ^d	6.50±0.31 ^c	7.50±0.38 ^a	7.00±0.46 ^b	6.49±0.42 ^c
Survival (%)	56.6±8.4 ^d	66.6±5.9 ^{bc}	80.0±6.3 ^a	70.0±8.4 ^b	60.0±5.1 ^{cd}
Total feed given (g)	280.89	412.49	745.49	511.08	346.17
Apparent feed conversion ratio	2.38±0.12 ^a	1.98±0.06 ^b	1.88±0.07 ^c	1.84±0.09 ^c	2.02±0.13 ^b
Protein efficiency ratio	0.99±0.02 ^d	1.28±0.07 ^c	1.44±0.02 ^a	1.43±0.03 ^{ab}	1.41±0.02 ^b
<i>Juveniles</i>					
Initial body weight (g)	7.15±0.26 ^a	7.15±0.26 ^a	7.15±0.26 ^a	7.15±0.26 ^a	7.15±0.26 ^a
Final body weight (g)	14.27±3.2 ^d	18.52±2.9 ^c	26.64±3.3 ^a	21.08±2.8 ^b	19.56±3.2 ^{bc}
Net body weight gain (g)	7.12±3.0 ^d	11.37±2.4 ^c	19.49±3.1 ^a	13.93±2.4 ^b	12.41±3.0 ^{bc}
Specific growth rate (%bw d ⁻¹)	1.38±0.24 ^d	1.90±0.27 ^c	2.63±0.31 ^a	2.16±0.25 ^c	2.01±0.21 ^{bc}
Survival (%)	60.0±6.5 ^d	66.6±6.5 ^c	80.0±8.4 ^a	73.3±4.3 ^b	60.0±6.5 ^d
Total feed given (g)	80.82	107.31	132.60	94.89	96.75
Apparent feed conversion ratio	3.57±0.13 ^a	2.39±0.09 ^b	1.62±0.06 ^c	1.99±0.07 ^c	2.34±0.11 ^b
Protein efficiency ratio	0.74±0.04 ^d	1.04±0.09 ^c	1.67±0.05 ^a	1.47±0.06 ^{ab}	1.11±0.07 ^b

Values are mean ± standard error of means for random samples at a time, in three replicates, from each tank

Values with same superscript in a row do not differ significantly ($p>0.05$)

Table 4. Growth performance and feed utilisation of *M. rosenbergii* post-larvae and juvenile fed diets supplemented with the probiotic Prosol

Nutritional indices	Diet				
	F0	F1	F2	F3	F4
<i>Post- larvae</i>					
Initial body weight (g)	0.45±0.06 ^a	0.45±0.06 ^a	0.45±0.06 ^a	0.45±0.06 ^a	0.45±0.06 ^a
Final body weight (g)	5.89±1.12 ^d	8.94±1.70 ^{bc}	10.2±2.48 ^b	13.35±2.67 ^a	7.78±1.24 ^c
Net body weight gain (g)	5.44±1.72 ^d	8.49±2.02 ^{bc}	9.75±1.65 ^b	12.9±2.28 ^a	7.33±1.45 ^c
Specific growth rate (%bw d ⁻¹)	5.14±0.29 ^d	5.98±0.47 ^b	6.24±0.48 ^{ab}	6.78±0.76 ^a	5.70±0.52 ^c
Survival (%)	53.3±8.5 ^c	56.6±5.2 ^c	63.3±6.4 ^b	76.6±8.6 ^a	56.6±4.9 ^c
Total feed given (g)	214.92	362.82	442.38	635.43	288.83
Apparent feed conversion ratio	2.29±0.06 ^a	2.24±0.08 ^{ab}	2.19±0.04 ^{bc}	2.09±0.03 ^d	2.14±0.02 ^{cd}
Protein efficiency ratio	1.14±0.03 ^c	1.12±0.02 ^c	1.19±0.04 ^{bc}	1.33±0.02 ^a	1.23±0.03 ^{ab}
<i>Juvenile</i>					
Initial body weight (g)	7.26±0.48 ^a	7.26±0.48 ^a	7.26±0.48 ^a	7.26±0.48 ^a	7.26±0.48 ^a
Final body weight (g)	12.41±1.2 ^d	13.20±1.8 ^c	23.96±4.1 ^a	17.60±2.6 ^b	13.49±3.1 ^c
Net body weight gain (g)	5.15±0.8 ^d	5.94±0.9 ^c	16.70±3.75 ^a	10.34±2.1 ^b	6.23±1.7 ^c
Specific growth rate (%bw d ⁻¹)	1.07±0.06 ^d	1.20±0.12 ^c	2.39±0.32 ^a	1.77±0.13 ^b	1.24±0.17 ^c
Survival (%)	53.3±4.2 ^d	60.0±6.5 ^c	80.0±8.4 ^a	66.6±6.5 ^b	60.0±4.8 ^c
Total feed given (g)	66.36	77.28	117.11	104.13	74.06
Apparent feed conversion ratio	5.71±0.06 ^a	4.13±0.03 ^b	1.77±0.08 ^d	2.86±0.02 ^c	4.27±0.05 ^b
Protein efficiency ratio	0.55±0.02 ^d	0.65±0.02 ^c	1.63±0.05 ^a	0.99±0.04 ^b	0.72±0.03 ^c

Values are mean ± standard error of mean for random samples at a time, in three replicates, from each tank

Values with same superscript in a row do not differ significantly (p>0.05)

survival and growth performance of freshwater prawn post-larvae as well as juveniles. The administration of the probiotic might have significantly altered the proportion of *Lactobacillus* sp. composition in the gut flora, leading to exclusion of other harmful bacteria and thus resulting in enhanced survival of prawns. *Bacillus* is able to compete with other bacteria for nutrients and space and can exclude other bacteria through the production of antibiotic (Verschuere *et al.*, 2000). *Lactobacillus* spp. have been shown to produce digestive enzymes such as amylase, protease and lipase, which results in enhancing the concentration of intestinal digestive enzymes (Lee, 1990). The bacteria would have also improved digestive activity *via* synthesis of vitamins and co-factors or *via* enzymatic improvement (Gatesoupe, 1999). Gullian *et al.* (2004) demonstrated significant growth increase in shrimp inoculated with *Bacillus* sp. El-Dakar and Goher (2004) observed that enhanced growth was generally obtained in shrimp, fed with diets having *B. subtilis*. Ziaei-Nejad *et al.* (2006) observed increase in specific activities of the digestive enzymes in probiotics treatment, which lead to enhanced digestion and increased absorption of nutrients, and this in turn contributed to the improved survival and growth in *Fenneropenaeus indicus*. On the contrary, McIntosh *et al.* (2000) and Shariff *et al.* (2001) found that treatment of *Litopenaeus vannamei* and *Penaeus monodon*

with commercial *Bacillus* probiotic did not significantly increase (p>0.05) either survival or growth. This may be due to the differences between the species. Further, prawn fed on probiotic supplemented diets recorded reduced apparent feed conversion ratio and improved protein efficiency ratio. Saad *et al.* (2009) and Haroun *et al.* (2006) found similar effects of probiotic Biogen® supplementation on feed utilisation by *M. rosenbergii* and Nile tilapia. Uma *et al.* (1999) observed a significant improvement in FCR and PER of shrimp larvae when fed with *L. plantarum* bio-encapsulated *Artemia*. Ziaei-Nejad *et al.* (2006) also observed an increase in absorption of food in probiotics treatment, which contributed to improved FCR and SGR in *F. indicus*. Similar observations were also reported by Suralikar and Sahu (2001) when feeding probiotic, *L. cremoris* diet at 8.5 x 10 cfu g⁻¹ to post-larvae of *M. rosenbergii*.

From the results of the present study, it is evident that significant growth improvement and feed conversion ratio were recorded when probiotic Improval, derived from *Lactobacillus sporogenes* and *Saccharomyces cerevisiae*, at 4% were fed to post-larvae and juveniles of *M. rosenbergii* by dietary supplementation. Further research is needed to ascertain the mode of action of the probiotic supplement on feed digestibility in *M. rosenbergii* and its effect on immune response and stress resistance.

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