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Feed Additive Bioboost Forte: Influence on Growth and Muscle Composition of Freshwater Prawn, *Macrobrachium rosenbergii* (de Man 1879)

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Abstract

The effect of Bioboost forte, a commercial probiotic feed additive, containing *Saccharomyces cerevisiae* and *Bacillus coagulans*, was evaluated in *Macrobrachium rosenbergii* (de Man 1879) through an experiment of 105 days. Bioboost forte was added at 0, 25, 50, 75 and 100 $mg \cdot kg^{-1}$ to the basal diet having 35% protein, prepared by incorporating fish meal, groundnut oilcake, rice bran, tapioca flour and vitamin-mineral mixture. The test diets were fed to prawn juveniles of average weight 0.9 ± 0.04 g stocked in 25 m^3 outdoor cement tanks in triplicate. Addition of the probiotic enhanced body weight gain significantly ($P<0.05$), the best being with 75 mg. The highest feed conversion efficiency, protein efficiency ratio and RNA : DNA ratio was obtained in this treatment. The highest protein and fat content of prawn muscle as well as amylase and protease activity were also recorded in 75 mg Bioboost forte treated prawns. The results indicate the beneficial effects of adding Bioboost forte to the diet of *M. rosenbergii*.

Introduction

Use of growth-promoting agents in aquaculture has increased in the past decade due to the twin advantages of reduction in culture period and total feed cost. They fall under the categories of probiotics, prebiotics, antibiotics, inophores and salts; among them, probiotics have received greater attention. Dietary probiotics result in the best use of carbohydrates, protein and energy, thereby impacting growth positively (Li and Gatlin III 2004). According to Irianto and Austin (2002), probiotics may stimulate appetite and improve nutrition by the production of vitamins, detoxification of compounds in the diet, and by breakdown of indigestible components. Among the probable modes of action of probiotics are (i) competitive exclusion, inhibiting the colonization of potential pathogens in the digestive tract by the production of inhibitory compounds or through competition for nutrients and/or space, (ii) modification of microbial metabolism, and (iii) stimulation of host immunity responses.

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Probiotics improve feed utilization and growth performance in fish and shellfish (Tewary and Patra 2011; Mohapatra et al. 2012; Seenivasan et al. 2012; Gupta and Dhawan 2013; Prasad et al. 2013; Saini et al. 2014; Mona et al. 2015; Ghosh et al. 2016; Hussein et al. 2016; Mohammadi et al. 2016). Freshwater prawn *Macrobrachium rosenbergii* (de Man 1879), commonly known as scampi, enjoys immense potential for culture in India. Being a hardy species with omnivorous feeding habit, it is suitable for cultivation under monoculture as well as polyculture in tropical and subtropical climates. This investigation was undertaken to determine the effect of graded levels of Bioboost forte on growth, muscle composition and digestive enzyme activity in juveniles of *M. rosenbergii*.

Materials and Methods

Preparation of diets

Ingredients used for diet preparation were locally procured. They were sieved using ISI standard mesh No.1. Bioboost forte was obtained from Lyka Labs Ltd., Mumbai. Five isonitrogenous and isocaloric diets (T_0, T_1, T_2, T_3, T_4) were formulated incorporating 0, 25, 50, 75 and 100 mg of the additive, respectively (Table 1). Each diet was prepared separately according to Jayaram and Shetty (1981). The required quantity of finely ground ingredients was mixed with water adequate to prepare the moist dough which was cooked at 105 °C for 30 minutes and then cooled. Bioboost forte powder and vitamin and mineral mixture were added to the cooled dough and thoroughly mixed. The dough was then extruded through a pelleting machine to obtain pellets of 2 mm diameter. These pellets were dried to less than 10% moisture at 40 °C and stored at room temperature in air-tight containers until use.

Table 1. Level of inclusion of different ingredients in the experimental diets.

	Diets				
Ingredients (%)	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**
Fish meal	30.0	30.0	30.0	30.0	30.0
Groundnut cake	39.5	39.5	39.5	39.5	39.5
Rice bran	19.5	19.5	19.5	19.5	19.5
Tapioca flour	10.0	10.0	10.0	10.0	10.0
Vitamin-mineral mixture*	1.0	1.0	1.0	1.0	1.0
Biobooste forte (mg)**	0	25	50	75	100

*Nuvamin forte, **200 g of Biobooste forte consists of 50 g live yeast culture of *Saccharomyces cervisiae* and 6000 x 18 million CFU of *Bacillus coagulans*

Experimental set up

The growth experiment of 105-day duration was carried out in triplicate in 15 outdoor cement tanks of 25 m^3 (5x5x1m) each, without a soil base. PVC pipes and broken tiles were used as hide-outs for prawns in equal numbers covering 15% of the bottom of all tanks. The tanks were filled with water drawn from a nearby well and the height was maintained at 85±5 cm over the experimental duration. *Macrobrachium rosenbergii* post-larvae obtained from Rosen Fisheries, Trissur, Kerala were reared to juvenile stage in the College of Fisheries, Mangalore Fish Farm and acclimated to the experimental conditions. Juveniles of av. wt. 0.9±0.04 g were stocked in all the experimental tanks at a density of 50 per tank and were fed with the formulated diets every day at 10% of body weight during the first month and at 5% during the remaining period in the morning and evening in 2 equal portions. Feeding was done using plastic trays measuring 25x20x5 cm kept suspended in the tanks at a depth of about 60 cm. Prawns were sampled every 15 days to measure body weight and length. The quantity of feed given was re-adjusted after each sampling, based on the weight recorded.

Water analyses

Water samples collected from the experimental tanks on prawn sampling days between 09.00 and 10.00 AM were analysed for temperature, dissolved oxygen, pH, free carbon dioxide, total alkalinity and ammonia. Digital thermometer was used to record temperature, while digital pH meter (LI-120, ELICO, India) was used to measure pH. Dissolved oxygen, free carbon dioxide, total alkalinity and ammonia were determined following standard procedures (APHA 1992).

A net made of no. 30 bolting silk cloth having 60 µm mesh size was used to collect plankton samples at 15-day intervals, by filtering 100 L of water from different locations of each experimental tank. Dry weight of plankton was determined by drying the filtrate overnight at 80 ºC in an oven.

Muscle DNA and RNA

One gram of the muscle was taken and homogenized with 9 mL of 1% potassium hydroxide solution. To 0.5 mL of the homogenate same quantity of 1N perchloric acid (PCA) was added and kept in ice cold water bath for 30 minutes. It was then centrifuged at 3,000 rpm for 10 minutes and the supernatant was discarded. To the residue 3 mL of 10% PCA was added and kept in a water bath at 70 °C for 20 minutes. Later it was centrifuged at 3,000 rpm for 10 minutes and the supernatant was used for nucleic acid estimations. DNA content was determined by the diphenylamine method of Giles and Myres (1965), while RNA was estimated as described by Ceriotti (1955) using Orcinol reagent.

Proximate composition

Feed ingredients, diets and prawn muscle were subjected to proximate composition analyses. Muscle was collected from 3 prawns per replicate tank (9 per treatment) on termination of the experiment after removing the exoskeleton and it was pooled tank wise. Thus, samples were analysed in triplicate. Protein, fat, fibre and ash were determined according to the methods described in AOAC (1995). The 'Difference method' of Hastings (1976) was used to calculate the nitrogen-free extract (NFE). Protein, fat and carbohydrate (NFE) contents were multiplied by factors of 5 (Smith 1975), 9 and 4 (Hastings 1975) respectively to obtain the energy value which is denoted in $kJ \cdot g^{-1}$.

Digestive enzyme activity

On termination of the experiment, six prawns from each tank were sacrificed for digestive enzyme activity analyses. The hepatopancreas was dissected out and homogenized in ice cooled condition with distilled water (4 $mL \cdot g^{-1}$) and centrifuged at 16,000 rpm for 20 minutes at 4 ºC. The supernatant (crude enzyme extract) was stored at -20 ºC until further use. Amylase activity was measured using 1% starch as the substrate. The assay mixture contained 0.1 mL crude enzyme extract, 1.0 mL phosphate buffer plus 1.0 mL substrate and was incubated at 28 °C for 10 minutes. The resulting reducing sugars were determined following 3, 5-dinitro salicylic acid (DNS) method of Bernfeld (1955) using maltose as the standard. Total proteolytic activity was determined by the casein digestion method of Kunitz (1947). The assay mixture contained 0.1 mL crude enzyme extract plus 2.0 mL of casein buffer substrate and was incubated at 28 ºC for 15 minutes. The resulting tyrosine was determined using tyrosine as the standard.

Performance indices and statistical analysis

Performance indices viz. specific growth rate (SGR), feed conversion efficiency (FCE), protein efficiency ratio (PER), survival and net production were calculated as follows.

SGR (% day) = [(ln final weight-ln initial weight/Duration of the experiment in days)] x 100.

FCE (%) = Weight gain (g)/Feed consumed (g) x100.

PER = Increment in body weight (g)/Protein intake (g).

Survival (%) = Number of prawns alive on termination/Number of prawns stocked x 100.

Net production (g) = Final body weight (g) x Number of prawns harvested.

One-way analysis of variance (ANOVA) was used to compare various parameters among treatments, followed by Duncan's multiple range test at $P<0.05$ (Duncan 1955; Snedecor and Cochran 1968).

Results

The range of water quality parameters monitored over the experimental period was as follows. Temperature: 27.5 to 32.1 °C, pH: 7.0 to 8.8, dissolved oxygen: 4.56 to 10.87 ppm, free carbon dioxide: nil to 2.1 ppm, total alkalinity: 40 to 87 ppm and ammonia: 0.17 to 7.95 µg-at·L^{-1}. The average plankton dry weight values varied from 1.0 to 11.0 mg·100 L^{-1}, without major variation among treatments. Proximate composition of the experimental diets is shown in Table 2.

Table 2. Proximate composition of the control and Bioboost forte incorporated diets (Mean±S.E.).

Treatment	Parameter (%)						
	Moisture	Crude protein	Crude fat	Crude fibre	Ash	NFE	Caloric value (kJ·g^{-1})
T_0	5.23±0.03	35.09±0.09	4.87±0.04	9.58±0.05	15.98±0.05	29.25	14.07
T_1	6.60±0.07	35.07±0.10	4.79±0.05	9.21±0.12	15.29±0.09	29.04	14.00
T_2	6.50±0.05	35.04±0.06	4.83±0.02	8.99±0.11	16.21±0.10	28.43	13.91
T_3	5.82±0.08	35.09±0.08	4.80±0.06	8.69±0.08	16.12±0.12	29.48	14.09
T_4	6.16±0.12	35.02±0.06	4.86±0.05	8.74±0.10	16.19±0.08	29.03	14.01

The best prawn growth on termination of the experiment was observed in T_3 treatment (22.16 g), followed by T_4 (21.65 g), T_2 (17.19 g), T_1 (14.31 g) and T_0 (13.74 g) respectively. The difference in weight gain in T_3 and T_4 treatments was not significant; both were significantly higher than the rest of the treatments and the control. Prawn weight in T_2 treatment differed significantly from that of T_1 and the control. The average SGR ranged from 1.04% (T_0) to 1.24% (T_3). The lowest RNA:DNA ratio of 8.82 was obtained in the control; its values were higher in all treatment groups, the highest (13.15) being under T_3 treatment that recorded the best growth. Survival of prawns was lower in all the treatments, the average values ranging from 32% (T_0) to 37% (T_2). The highest net production was obtained in T_3 treatment (Table 3). FCE and PER were the best in T_3 treatment (36.03%, 1.02) and the least under T_1 treatment (26.15%, 0.74). Protein and fat contents of prawn muscle were the highest under T_3 treatment (20.75%, 2.91%) as against the lowest of the control (17.06%, 2.53%). Moisture varied from 66.53% in T_2 to 69.04% in T_0. Amylase as well as protease activity in the hepatopancreas was also higher ($P<0.05$) in prawn from T_3 treatment, with the lowest activity in the control (Table 3).

Table 3. Growth parameters, muscle composition and digestive enzyme activity (Mean±S.E.) in prawn fed experimental diets.

Parameter	Diets				
	T_0	T_1	T_2	T_3	T_4
Final weight (g)	13.74±0.21[a]	14.31±0.32[a]	17.19±0.26[b]	22.16±0.51[c]	21.65±0.44[c]
Final length (cm)	10.51±0.65[a]	10.96±0.34[a]	11.84±0.52[a]	12.95±0.46[a]	12.35±0.86[a]
Specific growth rate (%)	1.04±0.07[a]	1.06±0.03[a]	1.13±0.05[a]	1.24±0.02[b]	1.23±0.04[b]
Food conversion efficiency (%)	26.24± 0.26[a]	26.15±0.24[a]	31.48±0.52[a]	36.03±0.43[b]	34.37±0.06[a]
Protein efficiency ratio	0.79± 0.03[a]	0.74±0.02[a]	0.89±0.02[b]	1.02±0.05[c]	0.97±0.01[c]
RNA : DNA ratio	8.82±0.36[a]	9.17±0.22[a]	9.59±0.15[a]	13.15±0.18[b]	13.13±0.12[b]
Survival (%)	32±2.16[a]	33±1.12[a]	37±1.78[a]	35±1.42[a]	33±0.96[a]
Net production ($g \cdot 25m^{3} \cdot 105$ $days^{-1}$)	219.84± 5.14[a]	236.12±4.15[a]	263.03±8.36[b]	387.80±4.31[d]	357.23±3.98[c]
Muscle proximate composition (%)					
Protein	17.06±0.02[a]	18.55±0.12[a]	18.95±0.07[a]	20.75±0.05[b]	18.08±0.06[a]
Fat	2.53±0.06[a]	2.70±0.02[a]	2.78±0.04[a]	2.91±0.03[b]	2.55±0.03[a]
Ash	3.96±0.5[a]	4.04±0.08[a]	4.22±0.03[b]	4.69±0.02[c]	4.78±0.04[c]
Calorific value($kJ \cdot g^{1}$)	5.87	6.05	6.27	6.28	6.08
Digestive enzyme activity* in hepatopancreas					
Amylase	79.76±0.14[a]	79.88±0.02[a]	82.14±0.18[b]	101.23±0.23[d]	90.90±0.11[c]
Protease	52.99±0.01[a]	61.73±0.02[b]	72.65±0.02[c]	85.25±0.02[d]	83.45±0.01[d]

Values in the same row with different superscripts are significantly (P<0.05) different

*****Mean total activity (units·g $tissue^{-1}$± S.E.)

Discussion

There was no significant variation in the quality of water among different treatments. The water temperature recorded in this study (27.5 to 32.1 ºC) was within the range of 25-32 ºC suggested for prawn culture by New (2002), whereas pH varied from 7.0 to 8.8, being in the acceptable range (Santosh and Singh 2007). The dissolved oxygen content ranged from 4.56 to 10.87 ppm in the different treatments and was not a limiting factor. Free carbon dioxide when detected was found at low levels, the highest being 2.1 ppm. According to Santhosh and Singh (2007), free carbon dioxide in culture water should be less than 5 $mg \cdot L^{-1}$. Alkalinity between 75 to 200 $mg \cdot L^{-1}$, but not less than 20 $mg \cdot L^{-1}$ is ideal for aquaculture (Wurts and Durborow 1992). Alkalinity of 40 to 87 ppm was recorded in the present study. Stone and Thomforde (2004) stated that less than 4 $mg \cdot L^{-1}$ is the acceptable level of total NH3-N, while Bhatnagar and Singh (2010) recommended <0.2 $mg \cdot L^{-1}$ of ammonia as suitable for pond fishery. Ammonia recorded in the present study was low, ranging from 0.17 to 7.95 µg-$at \cdot L^{-1}$. Plankton dry weight variation among the treatments being not significantly different, their contribution to prawn growth may be considered as equal in all the treatments.

Significantly ($P<0.05$) higher growth was observed in prawn fed diets T_2-T_4 than those under T_1 treatment and control; the best growth and SGR were recorded in T_3 treatment. The difference in weight gain and SGR of prawn from T_3 and T_4 treatments was not significant (Table 3). This shows that Bioboost forte at 75 $mg \cdot kg^{-1}$ diet is effective in inducing significantly higher growth in prawn. It is presumed that prawns were able to feed to satiation since the diets were water stable for more than 1 h. In *M. rosenbergii*, both yeast and bacteria have beneficial effects as probiotics, either alone or in combination (Gupta and Dhawan 2013; Prasad et al. 2013; Seenivasan et al. 2015, 2016). *Saccharomyces cerevisiae* and *Bacillus coagulans* in Bioboost forte might have acted synergistically to bring about improved growth of the treated prawn. *Macrobrachium rosenbergii* fed on *Lactobacillus* spp. (Suralikar and Sahu 2001; Venkat et al. 2004) and *M. amazonicum* fed with *S. cerevisiae* and yeast derivatives (Hisano et al. 2008) exhibited improved growth. Two *Bacillus* isolates from *M. rosenbergii* when included in its feed either alone or in combination enhanced growth. Also, both isolates were found to produce amylase and protease (Deeseenthum et al. 2007). Seenivasan et al. (2016) reported increased amino acid production, PER and growth in *M. rosenbergii* fed *S. cerevisiae* incorporated diets. Ghosh et al. (2016) reported significant impact of commercial probiotic Zymetin composed mainly of *Streptococcus faecalis, Clostridium butyricum and Bacillus mesentericus* on the growth of *M. rosenbergii*. Ziaei-Nejad et al. (2006) in *Fenneropenaeus indicus* (H. Milne Edwards 1837) and Boonthai et al. (2011) in *Penaeus monodon* Fabricius 1798 reported enhanced growth following feeding with *Bacillus* spp. supplemented diets.

Unlike growth, survival of prawns was not influenced by Bioboost forte. It remained low in all the treatments. Dietary supplementation of yeast, *S. cerevisiae*, improved growth and feed utilization in Israeli carp (Noh et al. 1994), Nile tilapia (Abdel-Tawwab et al. 2008), koi carp (Dhanaraj et al. 2010) and African catfish (Mona et al. 2015). Heidarieh et al. (2013) reported higher feed intake, feed conversion and growth performance in rainbow trout fed fermented *S. cerevisiae*. Supplementation of diet with probiotics significantly improved feed utilization, growth and survival of *Channa striata* (Bloch 1793) fingerlings (Munir et al. 2016). Bagheri et al. (2008) reported significantly higher SGR, PER and condition factor in rainbow trout fry fed probiotics (*Bacillus* spp.) supplemented diet during the two months of first feeding. According to Sanders et al. (2003), *Bacillus* species produce antibiotics, amino acids and enzymes that have positive nutritional effects. *Catla catla* (Hamilton 1822) fed probiotics *Lactobacillus plantarum* and *Bacillus megaterium* supplemented diets showed improved growth performance (Parthasarathy and Ravi 2011), whereas *Lactococcus lactis* and *Bacillus subtilis* resulted in maximum growth performance in rohu *Labeo rohita* (Hamilton 1822) fingerlings (Mohapatra et al. 2012). Common carp receiving probiotic (Biogen®) containing *Bacillus subtilis* through diet exhibited higher feed utilization, growth and body protein (Hussein et al. 2016). Improved growth, survival, body protein and digestive enzymes in sea bass was reported by Hamza et al. (2016) following dietary treatment of *Virgibacillus proomii* and *Bacillus mojavensis* either singly or in combination.

Feed conversion efficiency was superior in the treated prawns than the control; the best conversion was obtained under T_3 treatment. This must have been the result of better utilization of nutrients. The highest RNA:DNA ratio was also recorded in this treatment, which can be correlated with increased protein synthesis. RNA : DNA ratios have usually been related to the tissue growth rate (Perago´n et al. 2000). Increase in muscle is a product of protein accretion and cell proliferation. Proximate composition of prawn muscle was positively affected by Bioboost forte. Prawns from T_3 treatment recorded the highest protein and fat levels. Probiotics have been shown to improve biochemical composition in *M. rosenbergii* (Saad et al. 2009; Lara-Flores et al. 2010; Seenivasan et al. 2012) and also in Nile tilapia (Abdel-Tawwab et al. 2008) and African catfish (Mona et al. 2015). Khalil et al. (2012) observed improved nutrient utilization as well as carcass composition in tilapia fed yeast incorporated diets. Abdel-Tawwab et al. (2006) opined that changes in protein and lipid content in tilapia could be linked with changes in their synthesis, deposition rate in muscle and/or different growth rate.

Amylase and protease activities were higher in the treated prawns. This indicates that the digestive process was positively influenced by the feed additive. According to Furne et al. (2005), the digestion of food and absorption of nutrients depend on the availability and efficiency of digestive enzymes. Probiotics are known to aid digestion by exoenzyme supply and establishment of beneficial microflora in the digestive tract (Sankar et al. 2016). Seenivasan et al. (2016) observed significantly higher levels of digestive enzymes in *M. rosenergii* fed probiotics incorporated diets. Probiotic strains synthesize extracellular enzymes and also provide growth factors like vitamins, fatty acids and amino acids which benefit the digestive processes of aquatic animals (Balcázar et al. 2006). Probiotic yeast *Debaryomyces hansenii* HF1 secretes amylase and trypsin enzymes that aid digestion in sea bass larvae (Tovar et al. 2002). According to Haroun et al. (2006), nutrients are absorbed more efficiently when the feed is supplemented with probiotics. Ziaei-Nejad et al. (2006) recorded increase in specific activities of the digestive enzymes in probiotics (*Bacillus* spp.) fed *Fenneropenaeus indicus*, which lead to enhanced digestion and increased absorption of nutrients, and this in turn improved growth.

Conclusion

It is concluded that Bioboost forte is useful in the diet at a level of 75 $mg \cdot kg^{-1}$ in enhancing the growth performance of *M. rosenbergii*. Using this probiotic through diet could improve economic efficiency of prawn farming.

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