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EFFECT OF COMMERCIAL PROBIOTIC SUPPLEMENTATION ON GROWTH AND SURVIVAL OF POST-LARVAE OF *MACROBRACHIUM GANGETICUM* (BATE)

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ABSTRACT – Post-larvae of *Macrobrachium gangeticum* were fed a diet supplemented with commercial probiotic (Problend) containing *Lactobacillus sporogenes*, *Lactobacillus acidophilus*, *Bacillus Bacillus subtilis*, *Bacillus licheniformis, Sacharomyces cerevisiae*, seaweed extract, amylase, phytase, protease, cellulase, â-galactosidase, lipase, coated vitamin C, thiamine mononitrate, vitamin B_6, vitamin E and sodium benzoate for 90 days @ 10% of their body mass. The probiotic was incorporated @ 0.8% in the experimental diet. The results indicated a significant ($P < 0.05$) increase in body weight of post-larvae during 3 months of rearing on feed with probiotic as compared to those kept on control diet. The specific growth rate (SGR) and feed conversion ratio (FCR) of the post-larvae on control and experimental diets were 0.88 and 1.39 as well as 3.66 and 2.35, respectively.

***Key words* :** Dietary probiotic supplementation, growth, survival, post-larvae, *Macrobrachium gangeticum*.

INTRODUCTION

Over the last two decades, the shrimp farming has been hampered seriously due to outbreak of white spot disease (WSD) causing significant loss to aquaculture production and foreign exchange earnings (Kutty, 2005; New, 2005). Freshwater prawn farming is considered as an alternative to shrimp farming. Among freshwater prawns, *Macrobrachium gangeticum* is the third largest growing species, which attain maximum length and weight (male 250 mm and 100 mg; females 200 mm and 75 gm) in the Ganges and Brahmaputra riverine system (Kanaujia *et al,* 2005). Probiotic is cultured product or live microbial supplement when administered via feed, immersion or by injection in adequate amounts confer a health benefit on the host (Fuller, 1989; Irianto and Austin, 2002; Rengpipat, 2005; Denev *et al*, 2009; Dharmaraj and Kandasamy, 2010). Probiotics are commonly consumed as part of fermented foods with specially added active live cultures such as in yogurt, soy-yogurt or as dietary supplement (Balcazar *et al*, 2006; Yousefian and Amiri, 2009). The use of probiotics in aquaculture has tremendous scope and glorious future (Chen *et al*, 1992; Moriarty, 1997; Velmurugan and Rajagopal, 2009). Lactic acid bacteria (LAB) and bifidobacteria are the most common types of microbes used as probiotics but certain yeasts and bacilli may also be helpful (Vijayakumaran, 2001; Balcazar *et al*, 2006; Deeseenthum *et al*, 2007; Yousefian and Amiri, 2009). Recently, the use of probiotics to maintain healthy environment and improve production has been advocated but the observations are inconsistent and warrant further work for beneficial applications in prawn aquaculture (Dalmin *et al*, 2001; Ninawe and Selvin, 2009; Rahman *et al*, 2009; Yousefian and Amiri, 2009; Habib, 2010; Ngo and Fotedar, 2010; Nimrat and Vuthiphandchai, 2011). Therefore, an attempt was made to record the effect of a commercial probiotic (Problend, powder form) on post-larval growth and survival of *M. gangeticum* under hatchery conditions.

MATERIAL AND METHODS

The study was carried out at Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar (Orissa) during June-August 2010 in triplicate in six number of 500L FRP tanks. Stocking was done with uniform sized post-larvae (average weight 0.12 gm) @ 100 PL/m² in each tank. Two diets - one without probiotic (control, T-1) and other with probiotic (test, T-2) were given to the post-larvae of *M. gangeticum* for 90 days. The control feed (T-1) was prepared using ingredients like rice bran, groundnut oilcake, soyabean oilcake, fish meal, prawn meal, starch and vitamin-mineral (Table 1). They were mixed together with vegetable oil and adequate quantity of water to get homogenous dough, pelletized (2.4 mm dia) and dried. The dietary probiotic "Problend" (GlaxoSmithKline Pharmaceuticals Limited, Mumbai) composed of *Lactobacillus sporogenes* 90,000 million cfu, *Lactobacillus acidophilus* 45,000 million cfu, *Bacillus subtilis* 30,000 million cfu, *Bacillus licheniformis* 30,000 million cfu, *Saccharomyces cerevisiae* 125,000 millon cfu, seaweed extract 100 gm, amylase 24,000 IU, phytase 22,00,000 IU, protease 400,00,000 IU, cellulase 150-250 IU, â-galactosidase 800-1000 IU, lipase 50-100 IU, coated vitamin C 35 gm, thiamin mononitrate 1 gm, vitamin B_6 1 gm, vitamin E, 5,000 IU and sodium benzoate 6 gm (per kg) was procured, premixed with this feed @ 0.8% and cod liver oil was used for binding probiotic in the test feed (T-2). Both the feeds were provided to the post-larvae of *M. gangeticun* daily twice @ 10% biomass. Proximate analysis of feed was done following standard methods (AQAC, 1984). Tanks were cleaned daily and water was exchanged @50% twice every week. Average growth of PL was recorded at end of each month taking a minimum sample of 30 specimens. The water quality parameters temperature, pH, dissolved oxygen (DO), total alkalinity, total hardness and dissolved ammonia were monitored at regular intervals by following methods given in APHA (1999).

The specific growth rate (SGR) was calculated as:

$$SGR = \frac{(W_2 - W_1)}{} \times 100$$

where W_1 is initial mean weight, W_2 final mean weight and T time (days)

Feed conversion ratio (FCR) was estimated as:

$$FCR = \frac{\text{Total feed consumed (g)}}{\text{Total weight gain of post-larvae}}$$

The growth parameter was evaluated for statistical significance by using ANOVA (SAS, ver. 9.2) and Students "t" tests.

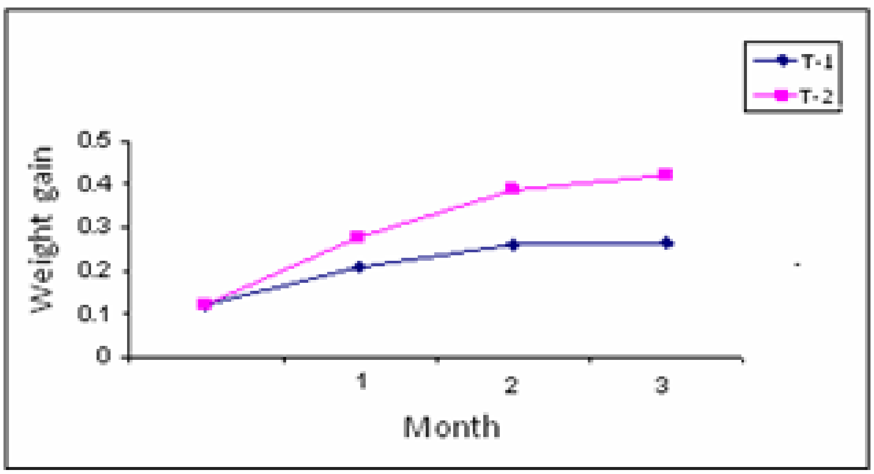

Fig. 1 : Growth performance of *M. gangeticum* on control (T-1) and test (T-2) diets.

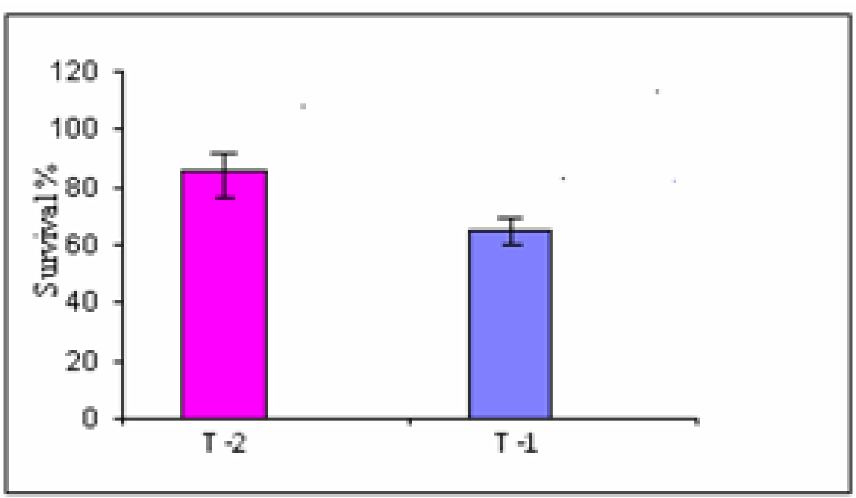

Fig. 2 : Survival percentage of *M. gangeticum* on control (T-1) and test (T-2) diet.

RESULTS AND DISCUSSION

Water quality parameters of tanks were within the optimal range (temperature 24.8-28.30C, pH 7.6-7.9, DO 4.3-4.5 ppm, total alkalinity 82-87 ppm, ammonia 0.083-0.085 ppm) of larval rearing. Growth of the post-larvae of *M. gangeticum* maintained on the control (T-1) and test (T-2) diets has been summarized in Table 2 and Fig. 1. Post-larvae kept on the test diet (T-2) recorded significantly ($P < 0.05$) higher growth than those maintained on control diet (T-1) (Table 3). The weight gain percentage in post-larvae on control diet (T-1) was 120% whereas in the test diet (T-2), it registered 251%. Growth increment by test diet (T-2) was significantly higher in prawns ($P < 0.05$) than those fed on control diet (T-2) (Table 3, Fig. 1). The specific growth rate (SGR) value in the post-larvae on test diet was 1.39 against 0.88 in control diet. Feed conversion ratio (FCR) reflecting the growth was 2.35 and 3.60 in the post-larvae kept in the test and control diet, respectively. Survival rate of the prawn in control (T-1) and test diet (T-2) was 65% and 85%, respectively (Fig. 2).

Though mode of the action of probioitcs has not yet been explored in details, they benefit the host by (i) competitive exclusion of pathogenic bacteria, (ii) source of nutrients and enzymatic contribution to digestion, (iii) direct uptake of dissolved organic material mediated by bacteria, (iv) enhancement of the immune response against pathogenic microorganisms and (v) anti-viral effects (Balcazar *et al*, 2006; Li *et al*, 2007; Yousefian and Amiri, 2009). Ravi *et al* (1998) reported the benefits of probiotics in maintaining water quality and enhancing growth rate in *Penaeus indicus*. As shrimp aquaculture production of the world has been decreased by the diseases caused by luminous *Vibrio* and/or viruses, probiotic technology provides a solution to these problems by adding selected bacterial species to displace deleterious bacteria in large aquaculture ponds. Abundance of virulent luminous *Vibrio* strains can be controlled when specially selected probiotic strains of *Bacillus* species are added in ponds.

Rengpiat *et al* (1998) and Rengpiat (2005) found significantly higher growth of post-larvae of farmed black tiger shrimp (*Penaeus monodon*) with dietary probiotics. During proliferation, *Lactobacillus* produces biologically active lactic acid maintaining intestinal pH in the range of 5.5-7.0 (Vieira *et al*, 2008; Yousefian and Amiri, 2009). The spore of *Bacillus* species is especially easy to introduce in dry foods and/or *Artemia* (Austin and Allen, 1982; Sugita *et al*, 1998). Improvement in growth of the giant freshwater prawn, *Macrobrachium rosenbergii* has been recorded by feeding *Bacillus* spp. as probiotic bacteria (Deeseenthum *et al,* 2007). In the present study, improved feed conversion was observed in post-larvae of *M. gangeticum* maintained on test diet over the control. Better feed utilization efficiency consequent to dietary administration of non-hormonal growth promoter has been reported by Ahmad and Matty (1989). The probiotic administered might enhance growth by increasing availability of nutrients by change in gut microflora or its absorptive capacity (Moriarty, 1999; Irianto and Austin, 2002; Balcazar *et al*, 2006; Yousefian and Amiri, 2009; Velmurugan and Rajagopal, 2009). Higher growth is also due to improve feed conversion ratio indicating better utilization of nutrients by the post-larvae of *M. gangeticum*. Lakshmanan and Sundarapandian (2008), Sundarapandian and Babu (2010), Soundarpandian *et al* (2010 and Rajanikant *et al.* (2010) have also observed better growth of *Penaus monodon* in hatchery and grow-out conditions given probiotic treatments. Bogut *et al* (1998) reported improved feed utilization in aquatic organisms fed probiotics probably indicating enhancement in digestive ability of the epithelium of digestive tract.

Survival of post-larvae of *M. gangeticum* kept on test diet (T-2) was 82% as against 62% in prawns kept on control diet (T-1) which may probably be due to enhancement of the immune response against pathogenic microorganisms (Rengpipat, 2005; Balcazar *et al*, 2006;

Table 1: Feed ingredients and proximate composition of the control (T-1) diet.

Ingredients	Percentage (%)	Proximate composition	Percentage (%)
Rice bran	40	Moisture	5.60
Groundnut oilcake	10	Protein	42.00
Soyabean oilcake	10	Fat	4.85
Fish meal	10	Ash	13.70
Prawn meal	18	Carbohydrate	33.92
Starch	10		
Vitamin-mineral mixture	2		

Table 2 : Growth data of *M. gangeticum* fed on control (T-1) and test (T-2) diet.

Days weight (gm)	Control diet (T-1)	Test diet (T-2) weight (gm)
0 days	0.12 ± 0.029	0.12 ± 0.029
30	0.1986667 ± 0.0176995	0.3593333* ± 0.0216871
60	0.2050000 ± 0.0151904	0.3653333 * ± 0.0239383
90	0.1840000 ± 0.0117209	0.3646667* ± 0.0286426

Values are means ± S.D. of 30 specimens. *Significant response: $P<0.05$

Table 3 : Comparison of growth of *M. gangeticum* on control and test diet.

Source	Sum of Square	Mean Square	F Value	Pr > F
Days	0.00354	0.00177	0.14	0.869
Treat	1.25835	1.25835	99.83	<.0001
Error	2.21836	0.01260		
Corrected	3.48025			

Yousefian and Amiri, 2009; Ngo and Fotedar, 2010). Lakshmanan and Sundarapandian (2008), Sundarapandian and Babu (2010), Soundarpandian *et al* (2010 and Rajanikant *et al* (2010) have also observed higher survival rate of *Penaus monodon* given probiotic treatment under hatchery and grow-out conditions. It is evident from the present study that the probiotic (Problend) is efficient in promoting higher growth during post-larvae rearing of *M. gangeticum*. The water quality parameters play important role in growth and survival of the post-larvae of *M. gangeticum* which were kept at the optimum levels throughout the culture period (Mishra *et al*., 2011) and the variations in these parameters were negligible in the test as well as control tanks.

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