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Research Article

Effect of A Probiotic Product, Promarine on Growth Responses of Indian White Prawn, *Penaeus* (*Fenneropenaeus) Indicus*

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Abstract

In this study, the impact of a probiotic product, Promarine was evaluated on the growth responses of post larvae of Indian white prawn, *Fenneropenaeus indicus*. There were three treatments and a control in the study. Promarine was either separately administered through water at 15 ppm/m^3 (Treatment 1) and through a commercial feed containing 35% protein at a concentration of 0.5% (Treatment 2) or in combination through water and feed (Treatment 3). The control was maintained without administering Promarine. The experiment lasted for a period of 90 days in fiber glass tanks (200 liter). Higher growth rates were observed in juveniles grown on Promarine through feed (Treatment 2) and water (treatment 1) when compared with Treatment 3 and control. There were no significant differences in water ammonia and nitrite concentrations among the treatments. Promarine was found to enhance growth and survival of post larvae of *F. indicus.*

Keywords: Probiotics, shrimp postlarvae, *Bacillus subtilis, Fenneropenaeus indicus,* aquaculture biotechnology.

Özet

Probiyotik Ürün Olan Promarinenin Beyaz Hindistan Karidesinin, *Penaeus* (*Fenneropenaeus) Indicus* Büyümesine Etkisi

Bu çalışmada, probiyotik ürün olan promarinin beyaz Hint karidesi, *Fenneropenaeus indicus* larvalarının büyümesine etkisi değerlendirilmiştir. Çalışmada üç muamele ve bir kontrol kullanılmıştır. Promarine 15 ppm/m^3 su (Tedavi 1), % 0.5 konsantrasyonda % 35 protein ihtiva eden ticari yem (Tedavi 2) ya da su ve yem ile kombinasyon halinde (Tedavi 3) tatbik edilmiştir. Kontrolde Promarine tatbik edilmemiştir. Deney fiberglas tanklarda (200 litre) 90 günlük bir sürede yürütülmüştür. Kontrol grubu ve Tedavi 3 ile karşılaştırıldığında en yüksek büyüme oranları Promarine yem (Tedavi 2) ve suyla (Tedavi 1) verilen yavrularda gözlenmiştir. Tedaviler arasında suda amonyak ve nitrit konsantrasyonlarında önemli farklılıklar görülmedi. Promarinenin *F. indicus* post larva büyüme ve yaşamasını geliştirdiği bulunmuştur.

Anahtar kelimeler: Probiyotikler, karides postlarva, *Bacillus subtilis, Fenneropenaeus indicus*, su ürünleri, biyoteknoloji.

Introduction

During the last fifty years, world aquaculture has increased from less than a million ton in 1950s to 45.5 million tones by 2004 with an economic value of US$64.0 billion (FAO, 2006). In contrast, in Saudi Arabia the annual increase from 2007 to 2008 was 17.36% with a production of approximately 22,345.059 (FFC, 2008). Some studies noted that opportunistic

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bacteria such as *Vibrio* sp were the common disease agents in shrimp farming industry (Song et al., 1993; Liu *et al*., 2004 Chanratchakool *et al*., 1995; Alapide-Tendencia and Dureza, 1997). Therefore, some products such as immunostimulants and probiotic were used in shrimp farms as an alternative to antibiotics, an approach earning a lot of attention in recent years (Sakai, 1999; Farzanfar, 2006). Probiotics are useful microorganisms in improving health of the aquatic organisms and control diseases (Tseng *et al*., 2008). Several studies have shown the positive impacts of probiotics on growth and reduction of prevalence of disease causing pathogens such as *Vibrio* spp. (Suzuki *et al*., 1996; Villamil *et al*., 2003; Planas *et al*., 2006; Keysami *et al*., 2007; Farahi *et al*., 2011). Previous studies have noted the application of probiotics as a biological control agent for farmed marine organisms (Rengpipat et al., 2000, 2003; Farzanfar, 2006; Chiu, *et a*l., 2007; Tseng *et al*., 2008), pathogen inhibition (Vaseeharan and Ramasamy, 2003; Decamp *et al*., 2008) and improvement of shrimp growth performance (Rengpipat *et al*., 2003; Wang, 2007; Decamp *et al*., 2008; Liu *et al*., 2009). Although, the beneficial role of probiotics in aquaculture is well documented in the literature, the effects of a commercially available probiotic product, Promarine, on growth performance and survival of shrimps have not been studied in detail. Therefore, the present study evaluates the impacts of probiotic Promarine, which contains *Bacillus subtilis*, on survival and growth of the shrimp *Fenneropeneaus indicus*, an ideal candidate species for coastal aquaculture in Saudi Arabia.

Materials and Methods

Experimental Design

The experiment was conducted at the fish farming facility of the Faculty of Marine Sciences, in the Obhur campus, King Abdul Aziz University, Saudi Arabia. Healthy, Indian white shrimp (*F. indicus* H. Milne-Edwards, 1837), aged PL-9 were obtained from National Prawn Co. Al-Laith, Kingdom of Saudi Arabia and transported in plastic bags. The postlarvae (PL-9) were acclimatized in three fiberglass tanks (300-Liter capacity) until PL-15 with an average weight of 0.007 (weighed using a digital balance), and transferred to experimental tanks using a small net.

The tanks were cleaned and disinfected using chlorine before the start of the experiment. Twelve (12) cylindrical fiberglass tanks (200-Liter capacity) were supplied with clean, fresh seawater passed through a water filtration unit for a period of 90 days. Water flow rate to each tank was 1 L min^{-1} and waste water was drained from the central bottom. Each hose of the draining pipe had a small nylon net (200-micron) to prevent the escape of shrimp postlarvae from the tanks. Seawater was recycled through a close system which consisted of one sand filter, one biological filter and one receiving tank. Tank water was aerated using a mechanical blower. The experimental protocol was a completely randomized design with four treatments; in treatment 1 (T-1) Promarine was added only to water, in treatment 2 (T-2) Promarine was added only to feed, in treatment 3 (T-3) Promarine was added to both water and feed, and in the control no probiotic treatment was the case.

Shrimp Feed and Probiotic

Shrimp were fed with the local diet manufactured at the fish farming facility of Faculty of Marine Sciences, King Abdul Aziz University. The commercial probiotic Promarine (Bio Solutions Co., Ltd. Thailand) was a multi-strain probiotic product containing population of four different *B. subtilis* strains.All treatments were formulated with Promarine at a concentration of 0.5% obtained

by dissolving in distilled water at 0.1 g ml^{-1} (5g probiotic per kg of diet, added to 50 ml distilled water giving a final concentration $5.01x10^{11}$ CFU of *Bacillus subtilis* per g of diet). Promarine and fish oil were added in diet after cooking. The ingredients and chemical composition of the diet used in the experiment were as described by National Prawn CO. AL-Laith, Kingdom of Saudi Arabia (Table 1). The ingredients were mixed, extruded, sun-dried and kept at - 20 °C. Sampling was done once in every 15 days with 20 % of the population sampled (five shrimp per tank randomly selected and weighed).

Final survival rates were calculated for each tank by counting the number of remaining shrimps and comparing it with the initial stock. At the end of the experiment, final body weight, final biomass, feed conversion ratio (FCR), average daily growth rate (ADGR) g/day, specific growth rate (SGR) % / day, feed efficiency ratio (FER), protein efficiency ratio (PER), daily feed intake (DFI) and daily protein intake (DPI) were calculated.

Physico-chemical characters of the water were measured every day between 11:00 am and 1:00 pm. Water temperature and dissolved oxygen content were checked using a DO meter (JENWAY 9015). Salinity was measured by a portable refractometer and pH was measured by a pH meter. Ammonia and nitrite were determined every week between 11:00 am and 1:30 pm using a UV mini spectrophotometer. Ammonia was measured by the method of Koroleff (1969) whereas nitrite concentration was determined using the Griess reaction as applied to seawater by Bendschneider and Robinson (1958).

Proximate chemical analysis of diet

Diet samples were analyzed for moisture and ash according to the methods AOAC (1990). Crude protein in the diet was estimated using Lowry et al. (1955) method as this

Table 1. Ingredients of feed (%) used for Indian white shrimp (*F. indicus*) culture.

Ingredients	Percentage
Fish meal	27
Soya bean	27
Barley	22.6
Corn	10.2
Sea weed	1.5
Wheat gluten	6.2
UF Binder	2.8
Yeast	2.7
Minerals and vitamins	
Monocalcium phosphate: 7 mg Kg^{-1}	
Mineral premix : 2.5 mg Kg^{-1}	
Vitamin premix : 5 mg Kg^{-1}	
Vitamin C : 2 mg Kg^{-1}	
Methionine: 0.4 mg Kg^{-1}	
Anti-mould: 1.5 mg Kg^{-1}	
Antioxidant : 1 mg Kg^{-1}	
Fish oil : 30 mg Kg^{-1}	
Lecithin: 40 mg Kg^{-1}	
Yeast : 5 mg Kg^{-1}	
UF Binder: 8 mg Kg^{-1}	
Seaweed: 5 mg Kg^{-1}	
Wheat gluten: 30 mg Kg^{-1}	

method was more sensitive than AOAC. The lipid content of the diet was estimated by Folch et al. (1955) method**.**

Statistical analysis

One-way analysis of variance (ANOVA; SPSS version 13) was used to find differences in terms of various parameters among the treatments. All data were compared using Duncan's multiple range tests. A significance level of ($P<0.05$) was used.

Results

Water quality

The mean values of the different water parameters observed in the culture tanks are shown in Table 2. No significant differences ($P < 0.05$) were observed in probiotic treatments compared with control. Ammonia concentrations were higher in the control compared with T-1, T-2 and T-3.

Growth Performances and Survival

Growth of *F. indicus* postlarvae in all treatments (T-1, T-2, T-3 and control) during the 90 days period of this study is described in Figure 1.

Promarine was found to enhance growth and survival of post larvae of *F. indicus* (Fig 2).

F. indicus with initial weight of 0.007 g, 0.0083g and 0.0075 g grew to a size of 5.66 g, 5.727 g and 4.206 g after 90 days in T-1, T-2 and T-3 respectively but in the control they did from 0.0083 g to 3.573g (Table 3). There were significant differences in specific growth rates among the treatments which were calculated as 7.3 % d^{-1}, 7.42 % d^{-1}, 7.18 % d^{-1} and 6.89 % d^{-1} for T-1, T-2, T-3 and control respectively. There were significant differences ($P < 0.05$) in final weight, average weight gain and daily growth rates between Promarine supplemented groups (T-1 and T-2) and control group (Table 3).

Table 2. Physico-chemical parameters of the water in the experimental tanks during the 90-day culture period. Mean and standard errors for three replicates are shown

Parameters	**Treatments**			
	Control	**Added to water only (T-1)**	**Added to feed only (T-2)**	**Added to water and feed (T-2)**
Ammonia (mg L^{-1})	0.0894 ±0.017	0.0823 ±0.012	0.0892 ±0.013	0.0865 ±0.017
Nitrite (mg L^{-1})	0.1411±0.02	0.1269±0.022	0.1415 ±0.021	0.1378±0.022
Temperature (°C)	29.58 ±0.117	29.63 ±0.116	29.61 ±0.113	29.59 ±0.122
Dissolved oxygen (mg L^{-1})	4.96 ±0.052	5.01 ±0.056	5.05 ±0.055	5.12 ±0.055
pH	8.229 ±0.012	8.2278 ±0.015	8.265 ±0.013	8.1898 ±0.011
Salinity	41.27 ±0.116	41.47 ±0.118	41.43 ±0.116	41.78 ±0.118

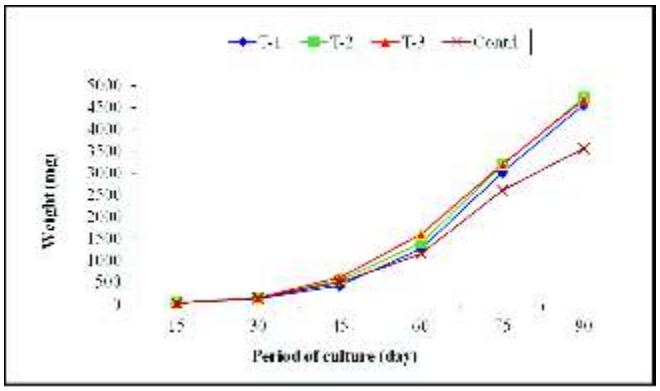

Figure 1. Growth of performance of *Fenneropenaeus indicus* differently treated with promarine, which contains *B. subtilis*. T-1= promarine added to water only, T-2= the promarine added to feed only, T-3= promarine added to both water and feed, control = without the probiotic treatment.

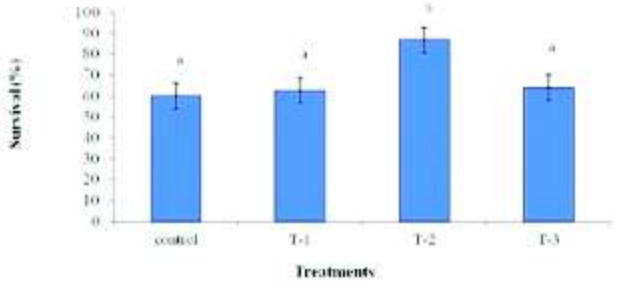

Figure 2. Survival rate (%) of *F. indicus* reared with Promarine added to water (T-1), feed (T-2) and both water and feed (T-3). Values are presented as mean ± SE, n =3. Means with the same superscript did not show significant variation.

Table 3. Effect of Promarine treatments (T-1, T-2 and T-3) on growth performance of postlarvae of *F. indicus* for a period of 12 weeks.

Experimental parameter	Treatments			
	Added to water (T-1)	**added to feed (T-2)**	**Added to feed And water (T-3)**	**Control**
Average Initial weight (g)	0.007 ±0.00005	0.0083 ±0.0002	0.0.0075±0.0001	0.008 ±0.0003
Average final weight (g)	5.66 ±0.196^{a}	5.727 ±0.109^{a}	4.207 ±0.196^{b}	3.573 ±0.226^{b}
Average Weight gain (g)	5.65 ±0.439^{a}	5.72 ±0.243^{a}	4.200 ±0.439^{b}	3.565 ±0.226^{b}
Final production (g m^{-2})	177.48 ±15.29^{a}	249.24 ±24.32^{b}	135.56 ±18.36^{ac}	109.05± 22.05^{c}
Daily growth rate (g d^{-1})	0.064 ±0.0050^{a}	0.0649 ±0.003^{a}	0.0477 ± 0.005^{b}	0.0406 ±0.003^{b}
Specific growth rate (% d^{-1})	7.30 ±0.078^{a}	7.42±0.060^{a}	7.18 ±0.130^{ab}	6.90 ± 0.137^{b}

Mean ± SE, n=15 for all means. Means followed by the same letter (s) are not significantly different (P > 0.05).

At the end experiment the survival rates in the T-1, T-2, T-3, and control groups were 62.67% ± 1.33, 86.67±5.33, 64 ±2.31, and 60± 8.327% respectively ((Figure 2). The survival rate of T-2 group was statistically different from other groups ($P < 0.05$; Figure 2).

Nutritional parameters

The daily feed intake and daily protein intake were determined as 0.64 g, 0.236 g in T-1, 0.673 g, 0.25 g in T-2, 0.65 g, 0.24 g in T-3, and 0.7 g, 0.26 g in control (Figures 3 and 4) .

There were significant differences ($P<0.05$) in daily feed intake and daily protein between T-1 and T-3 compared with T-2 and control (Fig. 3 and 4).

No significant differences were detected in protein efficiency ratio (PER), feed efficiency ratio (FER) and feed conversion ratio (FCR) of the postlarvae shrimp between control and T-1 and T-3 whereas significant differences ($P<0.05$) were observed between T-2 and control (Figures 5, 6 and 7).

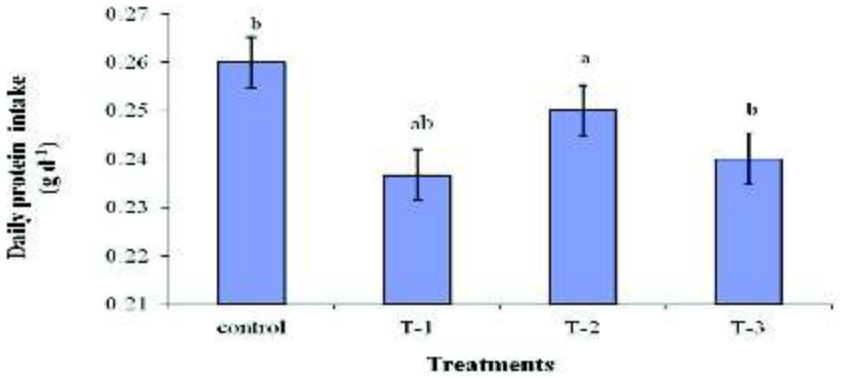

Figure 3. Daily protein intake (g d^{-1}) of *F. indicus* reared with Promarine added to water (T-1), feed (T-2) and both water and feed (T-3). Values are presented as mean ± SE, n =3. Means with the same superscript did not show significant variation.

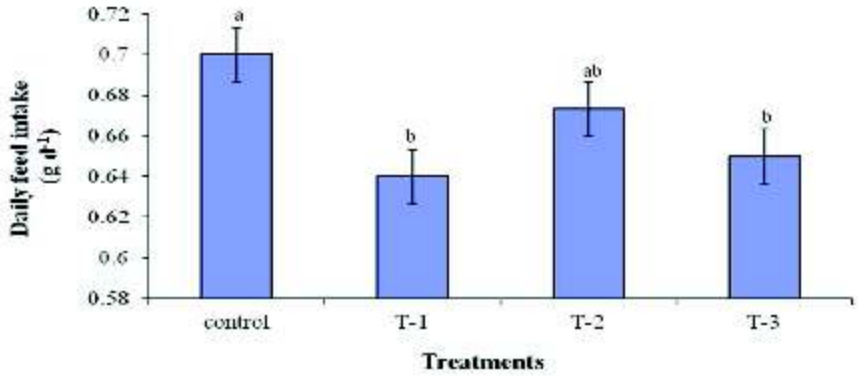

Figure 4. Daily feed intake (g d^{-1}) of *F. indicus* reared with Promarine added to water (T-1), feed (T-2) and both water and feed (T-3). Values are presented as mean ± SE, n =3. Means with the same superscript did not show significant variation.

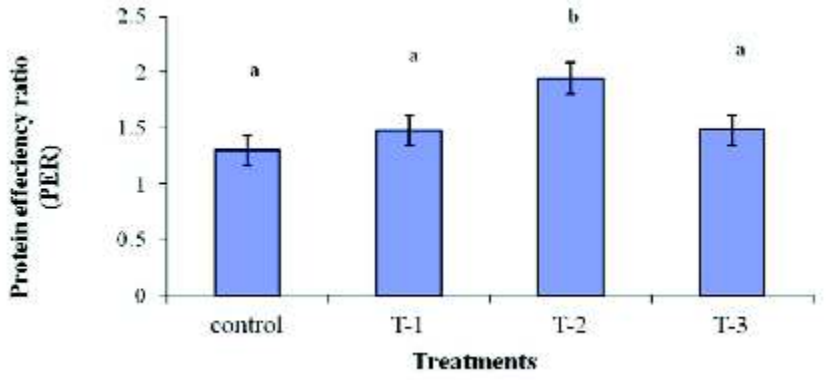

Figure 5. Protein efficiency ratio of *F. indicus* reared with Promarine added to water only (T-1), feed only (T-2) and both water and feed (T-3). Values are presented as mean ± SE, n =3. Means with the same superscript did not show significant variation.

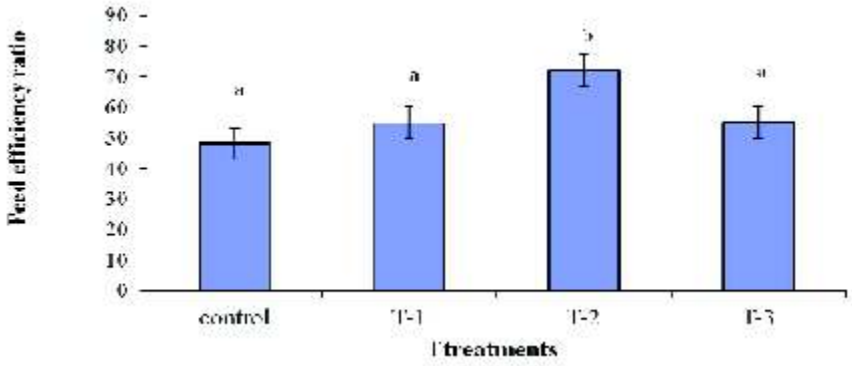

Figure 6. Feed efficiency ratio of *F. indicus* reared with Promarine added to water (T-1), feed (T-2) and both water and feed (T-3). Values are presented as mean ± SE, n =3. Means with the same superscript did not show significant variation.

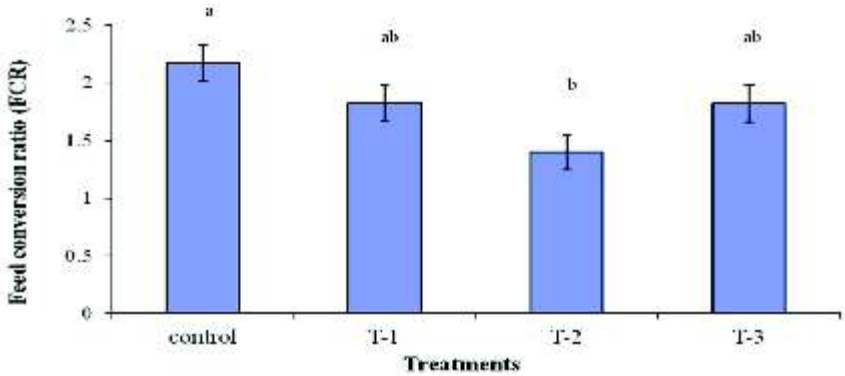

Figure 7. Feed conversion ratio (FCR) of *F. indicus* reared with Promarine added to water (T-1), feed (T-2)and both water and feed (T-3). Values are presented as mean ± SE, n =3. Means with the same superscript did not show significant variation.

Discussion

One of the most important factors affecting the shrimp production is the build up and toxicity of NH_3 with the intensification of culture. Wang *et al*. (2005) investigated the effect of commercial probiotics on water quality in *P. vannamei* ponds and showed that probiotics could significantly reduce the concentrations of nitrogen and phosphorus in pond water compared with the control. In the present study, use of a commercial probiotic, Promarine, in shrimp postlarvae showed no significant difference ($P > 0.05$) in water quality parameters of the treatment tanks compared with control (Table 2). Also as seen in Table 2, all the physicochemical parameters were well within the optimum levels recommended for shrimp culture (Chen *et al*., 1989; Chien, 1992; Boyd and Tucker, 1998). Thus, the results could indicate that good water quality and farming conditions were maintained in this study.

The results showed significant differences ($P < 0.05$) in survival between T-2 compared with T-1, T-3 (comparable to each other) and control. Overall, the findings indicated the enhancement of survival of shrimp particularly in dietary probiotic administered group at 0.5%. These observations are in agreement with many other studies on shrimp (McIntosh *et al*., 2000; Sheriff *et al*., 2001; Ziaei-Nejad *et al*., 2006) and fishes (Suzer *et al*., 2008).

Also results of this study were in accordance with the one conducted by Piyatiratitivorakul *et al*. (2002), who observed a positive effect of *Bacillus* sp. survival in shrimp larvae**.** Dietary probiotic effect of *B. subtilis* (10^5 CFU g^{-1}) on shrimp *Litopenaeus vannamei* was previously recorded by Balcazar *et al*.(2007) in a 28-day experiment. Balcazar *et al*. (2007) found that the treatment with *B. subtilis* UTM 126 increased survival from 48.25% in the control to 81.75 % possibly due to the antimicrobial activity of *B. subtilis* against pathogenic *Vibrio* species, including *V. alginolyticus, V. parahaemolyticus*, and *V. harveyi*. Therefore the increased survival rate observed in the present study may be due to the effect of Promarine on digestive tract by increasing the proportion of *Bacillus* strains in the gut flora which in turn may lead to an inhibition of pathogenic strains such as *Vibrio harveyi, Vibrio anguillarum, and Vibrio damsela* (Rengpipat *et al*., 2000; Chiu *et al*., 2007; Tseng *et al*., 2008). However, this speculation needs a further confirmation.

The present results also indicated that shrimp productivity was higher in T-2 compared with other treatments due to better growth rate and survival. This may be due to a production of exogenous enzymes by the probiotic bacteria that may make a small contribution to the total enzyme activity in the gut. Also, the presence of the probiotic may in some way stimulate endogenous enzymes produced by the shrimp. These comments were previous noted in a study conducted on Indian white shrimp, *F. indicus* (Ziaei-Nejad *et al*., 2006).

Thus, the observed increase in growth and nutritional parameters in *F. indicus*, including improved feed conversion ratio (FCR), specific growth rate (SGR), daily growth rate (g d^{-1}) and feed efficiency ratio (%) and protein efficiency ratio (PER) may be due to an increase in enzyme activities by the probiotic bacterial strains. We may suggest that due to the expected increase in specific activities of digestive enzymes in probiotic treatments may have led to enhanced digestion and increased absorption of food, which in turn contributed to the improved growth and nutritional parameters. In all probiotic treatments, there was a decrease in daily feed and daily protein intakes whereas daily growth rate, daily weight gain and total production for

postlarvae shrimp were increased when compared to control. Once again, these results may be used as an indicator of activity of digestive enzyme as indicated by other studies (Ueberschar, 1993, 1995: Ziaei-Nejad *et al*., 2006). Also there are many studies which showed a positive effect of probiotic on growth parameters because the Gram positive bacteria, particularly members of the genus *Bacillus*, do secrete a wide range of exoenzymes (Moriarty, 1999).

In conclusion, our findings confirm the beneficial effects of probiotic bacterial strains in shrimp larval culture. Since only survival and nutritional parameters were measured in the present study, the assumption of competitive exclusion by non-pathogenic *Bacillus subtilis* as the main source of beneficial role is based on previous reports. Unfortunately, we did not measure the microbial load in water column and gut as well as digestive enzymes activities. Therefore we suggest further studies using probiotics with all stages of *F. indicus* (larvae-postlarvae) along with measuring bacterial load and digestive enzymes activities. Yet, overall results indicate that use of *B. subtilis* in shrimp feeds can reduce mortality in *F. indicus* during larval culture and increase growth rate.

Acknowledgements

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