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Effect of a Commercial Probiotic, Ecomarine on the Growth Performances of Indian White Prawn, *Penaeus* (*Fenneropenaeus) indicus (*M.Edwards*)*

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The influence of a commercial probiotic, Ecomarine was tested on the growth performance of post larvae of Indian white prawn, *Fenneropenaeus indicus* for a period of 90 days in fiber glass tanks (500 liter). There were three treatments and a control for the study. Ecomarine was administered at 15 ppm through water (Treatment 1), a concentration of 5% was supplemented through a commercial feed containing 35% protein (Treatment 2) and a combined supplementation through water and feed (Treatment 3). A control was maintained without administering Ecomarine. Higher growth was observed in juveniles grown in tanks supplemented with Ecomarine through feed and water (Treatment 2) when compared with Treatment 1 and control. There was no significant difference in the ammonia and nitrite concentrations in water between treatments and control. Ecomarine found to enhance growth and survival of post larvae of *Fenneropenaeus indicus.*

Key words: White Prawn, Commercial probiotic, Ecomarine.

Fenneropenaeus indicus are widely distributed throughout the Indian Ocean from southern Africa to northern Australia and to all Southeast Asia and Middle East[1]. Recent studies on the aquaculture of this species have been undertaken in Middle East especially in two countries: the Kingdom of Saudi Arabia and the Islamic Republic Iran[2]. There are many studies have been shown that negative effects of antibiotic and chemicals, including the study conducted by[3-5], Chemicals to improve the quality and sustainability of aquaculture production have been seen as desirable[6-9]. The benefits of such supplements include improved feed value, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, antimutagenic and anticarcinogenic activity, growth-promoting factors, and increased immune response[10]. Probiotics in aquaculture have been shown to have several modes of action: competitive exclusion of pathogenic bacteria through the production of inhibitory compounds; improvement of water quality; enhancement of immune response of host species; and enhancement of nutrition of host species through the production of supplemental digestive enzymes[11].

A probiotic is generally defined as a live microbial food supplement, which improves the balance of the host animal's intestinal flora[12]. As reported by several authors *Bacillus spp* are commonly used widely as putative probiotic in animal nutrition which are *B. subtilis, B. licheniformis, B. polymyxa, B. laterosporus and B. circulans*, *B. coagulans* [3-14; 8-3]. Many studies have been evidenced that probiotics are mono or mixed of microbes, or their spores would be isolated from intestinal or haemolymph of organisms culture (shrimp or fish etc...) or sandy bottom ponds, or commercial probiotic (solid, liquid, powder or

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tablet) as ecomarine or protexin[14-17]. Probiotics are used as water additives or as feed supplements[18; 19]. Previous applications of probiotics have proved beneficial to the host by improving growth, survival and health[18;19]. Probiotics have been found to be useful in farmed animal growth[20-22] and as biological control agent of diseases of farmed animals[20; 8; 25]. The beneficial effects of probiotic use in shrimp aquaculture are in the biological control of disease through improved shrimp immunity[23; 24; 27], pathogen inhibition[21; 8] and shrimp growth performance[26,21, 13, 27]. Also[28] found that positive effect of *Lactobacillus* strains (JK-8 and JK-11) on removal of pathogens and nitrogens from contaminated shrimp farms[14] found that exhibited significant ($P<0.05$) increases in both survival (11–17% higher) and wet weight (8–22% higher) as compared to controls when probiotic *Bacillus spp* of *Fenneropenaeus indicus* and the feed conversion ratio, specific growth rate, and final production were slightly, but significantly ($P<0.05$), higher in shrimp receiving the probiotic than in control shrimp which had received no probiotic especially in stages Nauplius through PL_{30}). Although, beneficial effects of probiotics are well known in aquaculture, there has been little study about influence of Ecomarine on the growth and survival in *Fenneropenaeus indicus*, Therefore, a study has been conducted to evaluate the efficiency of Ecomarine on growth and survival of post larvae of *Fenneropenaeus indicus* an ideal candidate species for coastal aquaculture in Saudi Arabia.

MATERIALS AND METHODS

The experiment was conducted at the Fish farming Centre at Obhur of Faculty of Marine Sciences, King Abdul Aziz University, Saudi Arabia for a period of 90 days in fibre glass tanks. There were three treatments and a control. All treatments and controls were replicated thrice. Healthy post larvae of *F. indicus* were obtained from National Prawn Co. Al-Laith Kingdom of Saudi Arabia. Postlarvae were acclimatized in thee fiberglass tanks (300-Liter capacity) prior to the experiment and recorded the average weight before stocking them in tanks. At the end of the acclimation period, post larvae were fed a diet three times daily for one week. Post larvae were stocked at the rate of 25 PL per tank. Ecomarine (Bio Solutions Co., Ltd. Thailand) was administered at 15 ppm through water (Treatment 1), a concentration of 5% was supplemented through a commercial feed containing 35% protein (Treatment 2) and a combined supplementation of Ecomarine through water and feed (Treatment 3). A control was maintained without administering Ecomarine. Shrimp were fed with a supplementary feed containing 37% crude protein, the diet was manufactured at the fish farming of King Abdul Aziz University, Faculty of marine science, (Abhor) Saudi Arabia. Feeding rates in this study adjusted every two weeks, based on sampling estimates for individual average body weight. Feeding rations code of diet, size and shapes to use present study was shown in index 1. The postlarvae shrimp fed three times daily at 8:00 am, 2:00 pm and 10:00 pm with diet as 30%, 30% and 40% respectively. Each day, any remaining diets were collected by siphoning before feeding. Every third day and dried in an aluminum bags was previous known weight, in an oven at 120 °C for 12 h and used in the estimation of the food conversion ratio, each tank was partially cleaned and the Exchange (about 20%) of the tank depending on change water quality especially Physico-chemical characters. Sea water had been recycled to use of close system which consists of one sand filter, one biological filter and one received tank at the least time two hours before inlet to the culture tanks. The temperature was maintained at 29.62 ±0.037 °C, the salinity range of water in the tanks was 37–45 ‰ and DO range of water in the tanks was 3.7- 6.9 mg/ L.

The average amount of total *Bacillus spp* in Eco- Marine was $1.01x10^{14}$ cfu/g. In Treatment 1, Eco-marine was added to the water tanks at the concentration of 15 ppm per m^3 and in Treatment 2, Ecomarine suspension was added to the feed only at the concentration 5gm per kg feed. Whereas in Treatment 3, Ecomarine was added to the both water and feed at a rate 15 ppm for water and 5gm/ kg for feed. The control group which had not received Ecomarine either in feed or in water.

Sampling was done once in every 15 days and a minimum of 20 % of the population was sampled to evaluate the growth performance and feed conversion., Average daily growth rate (ADGR) was calculated as $= W_t - W_0 / T$. Where:

Wt and Wo are the mean weight of the shrimps at current time (t) and at the commencement of the experiment (0), respectively, and T is the rearing period in days. Specific growth rate (SGR) % / day was calculated as ($\ln W_t - \ln W_0$ / t) x 100. Where: Wt and W0 are the mean weight of the shrimps at current time (t) and at the commencement of the experiment (o), respectively, and t is the number of rearing days (day). Feed Efficiency ratio (FER) = ΔW X 100 / Fc. Where: Fc is the total dry feed consumed (g) and ΔW is the total wet weight gained (g). Protein Efficiency ratio (PER) = ΔW/ Pc. Where: ΔW is the total wet weight gained (g) and Pc is the total protein consumed. Daily feed intake (DFI) = FI X 100 / [(initial shrimp wt+ final shrimp wt + dead shrimp wt) /2X days fed]. Where: FI is the feed intake (dry matter). Daily Protein intake (DPI) = PI X 100 / [(initial shrimp wt+ final shrimp wt + dead shrimp wt) /2X days fed]. Where: PI is the protein intake (dry matter).

Physico-chemical characters of the water were measured everyday between 11:00 am and 1:00 pm. Water temperature and Dissolved oxygen content were checked by using DO meter. (Jenway 9015). Salinity and pH was measured by a portable refractometer and pH meter (martini instrument co, Ltd.). Ammonia and nitrite were measured once in every week between 11:00 am and 1:30 pm using UV mini 1240 spectrophotometer. Ammonia was determined by the method of (29) and Nitrite concentration was measured using the Griess reaction methiod[30].

Statistical analysis was done by using one-way analysis of variance (ANOVA; SPSS version 13) to find out the statistical difference of various parameters between treatment and Control groups. All data means were compared by using Duncan's multiple range tests. Regression analysis was also employed to find out the relation ship between factors and groups).

RESULTS

The effect of Ecomarine probiotic on Ammonia concentration in water is presented in Fig. 1. There was no significant difference between treatments and control (P > 0.05).

Nitrite concentrations of water were presented in Fig. 2. No significant difference was observed (P>0.05) between treatments but significant differences was found when compared with control (P<0.05).

PH value of water in Ecomarine treated tanks are presented in Fig. 3. No significant difference was observed (p<0.05) between treatments and control**.**

Fenneropenaeus indicus with initial weight of 0.008 gm, 0.0070gm and 0.008 gm grew to size of 4.573 gm, 4.720 gm and 4.693 gm after 90 days of culture in three treatments (T-1, T-2, T-3) respectively and the control with initial weight of 0.0083 gm grew to size of 3.573gm was shown in Table 2 .Specific growth rates in all treatments (T-1, T-2, T-3 and control) were calculated as 7.19 %/ d, 7.39 %/d, 7.25 %/d and 6.89 %/d, respectively. There are no significant differences (p<0.05) on specific growth rate (SGR) of the postlarvae shrimp between (T-1, T-3) compared with control whereas was found significant differences (<0.05) among T-3 compared with control (Table 2).

At the end of experiment, the average weight gain and daily growth rates were observed as 4.71, 4.69, 4.57, and 3.57g in treatments and control respectively. There is no significant differences (P>0.05) on average weight gain and daily growth rates between control and treatments. Regression analysis indicates that there is significant difference (P<0.05) in final weight beween tratetment and control. Higher survival rate was observed **(**72 % ±2.309**,** 70.67% ±8.743 and 64% ±10.066) in T-3, T-2 and T-1 respectively and the lower (60 %± 8.327) in the control. No

Table 1. Regression analysis of Eco Marine and Eco-marine effect on growth of *F. indicus*.

Type of probiotic	Treatments	Growth = A+B*(2 weeks)
control	Without probiotic	Growth = 0. 603 + 0.908 * (2weeks)
Ecomarine	Add to water only	Growth = 0.743 + 0.905 * (2weeks)
	Add to feed only	Growth = 0.781+ 0.919 * (2weeks)
	Add to both water and feed	Growth = 0.775 + 0.884 * (2weeks)

significant difference (P>0.05) was found between treatments and control. The daily feed intake and daily protein intake were determined as 0.6500 gm, 0.2400 gm in T-2, 0.6700 gm, 0.2467 gm in T-3, 0.6933 gm, 0.2567 gm in T-1 and 0.6933 gm, 0.26 gm for control, respectively. There is significant differences (P<0.05) on daily No significant differences (P>0.05) on feed conversion ratio (FCR), feed efficiency ratio (FER) and protein efficiency ratio (PER) was observed in between control and treatments.

It is important to provide shrimp with a healthy environment and probiotics has a great deal of potential[31]. Wang investigated the effect of commercial probiotics on water quality in shrimp[32], *P. vannamei*, ponds and the results showed that probiotics could significantly reduce the concentrations of nitrogen and phosphorus in pond water compared with the control. In this study, the use of Ecomarine showed inconsistent results. In contrast, there was no obvious effect of probiotic on the water quality during the study. This result may be explained by the good water quality and rearing conditions in this study in contrast to others. In fact, the pH values and concentrations of ammonium and Nitrite were stable and within

Table 2. Effect of Ecomarine on the growth performances and feed utilization of postlarvae for a period of 12 weeks

Parameters	Treatments			
	Water	Feed	Water and feed	Control
Average initial weight (g)	0.008 ±0.0003	0.007 ±0.0003	0.008 ±0.0003	0.008 ±0.0003
Average Final weight (g)	4.573 ±0.251^{ab}	4.693 ±0.070^{a}	4.720±0.115^{a}	3.573±0.101^{b}
Average weight gain (g)	4.565 ±0.561^{ab}	4.686 ±0.158^{a}	4.712 ±0.257^{a}	3.565±0.226^{b}
Final production (gm/m$^{2)}$	150.31 ±35.91^{a}	165.04 ±17.25^{a}	169.55 ±7.41^{a}	109.05± 22.05^{a}
Daily growth rate (g/d)	0.0519± 0.0064^{ab}	0.0532± 0.0018^{a}	0.0536 ±0.003^{a}	0.041 ±0.003^{b}
Specific growth rate (%/d)	7.19 ±0.118^{ab}	7.39 ±0.124^{a}	7.25 ±0.079^{ab}	6.90 ±0.137^{b}
Daily feed intake (g/d)	0.6933±.0088^{bc}	0.6500 ±0.0058^{a}	0.6700 ±0.0057^{ab}	0.693± 0.009^{c}
Daily protein intake (g/d)	0.2567±.00333^{ac}	0.2400±0.00000^{b}	0.2467±.00333^{ab}	0.260 ±0.006^{c}
Feed conversion ratio(FCR)	2.0533 ±0.364^{a}	1.6900± 0.178^{a}	1.6600 ±0.364^{a}	2.167 ±0.303^{a}
Feed efficiency ratio (%)	51.40 ±.85748^{a}	60.70 ±7.0402^{a}	60.24 ±1.5362^{a}	48.09±1.422^{a}
Protein efficiency ratio (PER)	1.3900 ±0.2103^{a}	1.6400 ± 0.1914^{a}	1.6300 ±0.0416^{a}	1.30 ±0.1882^{a}
Survival (%)	64.0000±10.066^{a}	70.6667±8.743^{a}	72.0000±2.309^{a}	60.00 ±8.327^{a}

Mean ± SE. indicated N= 3 for Survival (%); N=15 for all other means. Means followed by the same Letter (s) are not significantly different (P < 0.05).

acceptable ranges of shrimp culture[33; 34].

No significant difference on survival was observed between treatments when compared with control (p>0.05). However, increase in survival was observed in all treatments when compared to control. This results are in similar with the studies conducted by[35-36]. They found that treatment of *P. monodon* and *Litopenaeus vannamei* with a commercial *Bacillus* probiotic did not significantly increase (P >0.05) either survival or growth also[14] found that treatment of *Fenneropenaeus indicus* with a commercial *Bacillus* probiotic did not significantly increase (P > 0.05) either survival particularly had received the probiotic only in the farming stage (PL30-PL120).

The increased survival by shrimp may be due to change the proportion of *Bacillus* bacteria in the gut flora, thus that lead to exclusion of other bacteria (especially harmful bacteria) by probiotic. Generally in similar with study was conducted by[14] particularly had received the probiotic both nursery and farming stages (P<0.05). In addition, these findings were agreement study on *Sparus aurata* by[37]. The increased of shrimp survival in most treatments may be due to administration of the probiotic significantly changed the proportion of *Bacillus* bacteria in the gut flora, or may be due to exclusion of other bacteria (especially harmful bacteria) by the probiotic, particularly in the postlarval stages where the *Bacillus* bacteria were dominant. In *P. monodon*, *Bacillus*, used as a probiotic, was able to colonize both the culture

water and the shrimp digestive tract; the *Bacillus* also was able to replace *Vibrio spp*. In the gut of the shrimp, there by increasing shrimp survival[6]. *Bacillus* bacteria through are able to out-compete other bacteria through the production of antibiotics[18; 10].

Bacillus administration also has been shown to increase shrimp survival by enhancing resistance to pathogens by activating both cellular and humoral immune defenses in shrimp[25]. *Bacillus* surface antigens or their metabolites act as immunogens for shrimp by stimulating phagocytic activity of granulocytes[38]. Thus, in this study we observed increased of shrimp survival in most treatments particularly, in (T-2, T-3) that may be due to administration of the probiotic significantly changed the proportion of *Bacillus* bacteria in the gut flora, or may be due to exclusion of other bacteria (especially harmful bacteria) by the probiotic, also was manifested the studies which conducted by (Rengpipat *et al*., 1998). In *P. monodon*, *Bacillus*, used as a probiotic, was able to colonize both the culture water and the shrimp digestive tract; the *Bacillus* also was able to replace *Vibrio spp* in the gut of the shrimp, there by increasing shrimp survival. Also some studies was observed that *Bacillus* bacteria through are able to out-compete other bacteria through the production of antibiotics[18;10] also[32].

Shrimp immune responses increased with probiotic treatment as shown in some previous studies[23-25]. Generally, in this study was observed enhancement of survival particularly in T2 and T3. These finding agreements with many studies on shrimp[14; 35-36] and fishes[37]. Also this study was agreement with study which conducted by[39] particularly to use Ecomarine that found Survival both Eco Marine added in water and added in the diet performed higher survival rate comparing to the control treatment, the Eco Marine added in the diet treatment showing the highest survival rate at the end of the experiment. Due to this treatment has the highest growth rate and survival rate, it could provide high shrimp production. Therefore this increased survival may be to evidence or indicated to effect of Ecomarine probiotic on digestive tract through increase proportion of *Bacillus* bacteria in the gut flora which to lead inhibition of *Vibrio harveyi, Vibrio anguillarum, and Vibrio damsela* that because due to an increase in the immune response and disease resistance against *Vibrio spp* as agreement with studies conducted by[25; 24; 23]. Thus, it was observed that an increase of shrimp production in all treatments compared with control. In the same shrimp production, an increased in all treatments but it

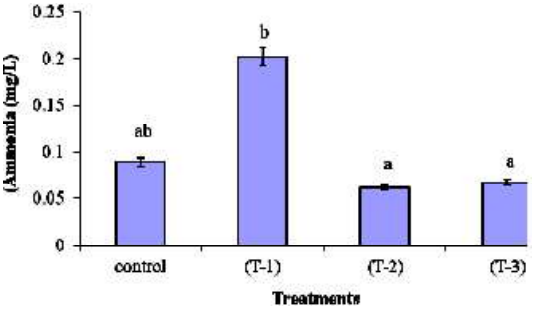

Fig. 1. Ammonia (mg L^{-1}) of water treated with EcomarineMean followed by the same letter (s) are not significantly different (P > 0.05)

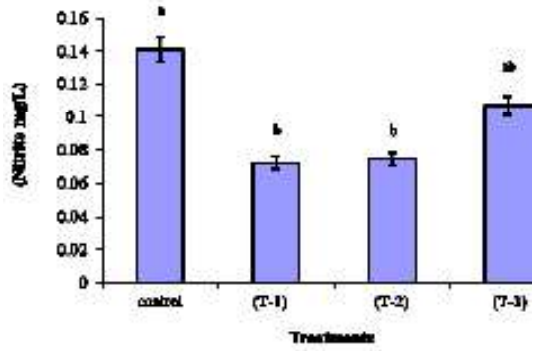

Fig. 2. Nitrite concentration (mg L^{-1}) of water treated with Ecomarine Means followed by the same letter (s) are not significantly different (P >0.05)

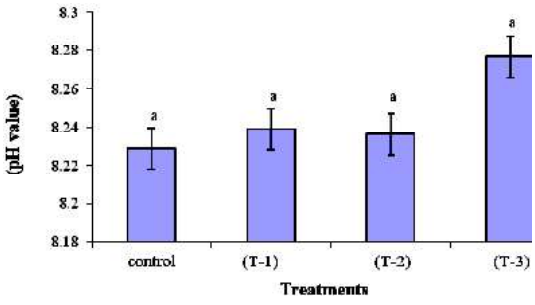

Fig. 3. The pH value of water treated with Ecomarine. Means followed by the same letter (s) are not significantly different (P < 0.05)

was no significant differences (P< 0.05) between all treatments and control. These findings were agreement with study on Indian white shrimp, *F. indicus*[14] particularly had received the probiotic both nursery and farming stages (P<0.05).shrimp *Penaeus monodon*[35].

Administration of the *Bacillus* bacteria to shrimp resulted in an increase in the specific activity of lipase, protease and amylase in the shrimp's digestive tract. Because gram-positive bacteria, particularly members of the genus *Bacillus*, do secrete a wide range of exoenzymes (40;18), We expected that the exogenous enzymes produced by the probiotic would contribute at most a small amount to the total enzyme activity of the gut. Perhaps, instead, the presence of the probiotic may in some way stimulate endogenous enzymes produced by the shrimp. However, the low proportion of *Bacillus* bacteria in the gut of shrimp in Experiment III (14). Thus, presence study the observed increases in growth and nutritional parameters in *F. indicus*, including improved feed Conversion ratio (FCR), specific growth rate (SGR), daily growth rate (g/d), daily feed intake (g/d), Daily protein intake (g/d), Feed efficiency ratio (%) and Protein efficiency ratio (PER) (table 5) in all treatments. We expected due to increases 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. Generally, in this study the observed increase in growth and nutritional parameters including average Final weight (g), average weight gain (g), Daily growth rate (g/d), Specific growth rate (%/d), Feed efficiency ratio (%) and Protein efficiency ratio (PER) in contrast, decrease in daily feed intake (g/d), Daily protein intake (g/d) and Feed conversion ratio(FCR).

There are increase significantly in growth and nutritional parameters including average Final weight (g), average weight gain (g), Daily growth rate (g/d), Specific growth rate (%/d) compared to control particularly when for their adding to feed only and both water and feed whereas was observed no significant differences (P<0.05) in Feed conversion ratio (FCR), Feed efficiency ratio (%) and Protein efficiency ratio (PER) compared to control. In contrast, sheriff *et al*., (2001) and Mclntosh *et al.,* (2000) found that treatment of *P. monodon* and *Litopenaeus vannamei* with a commercial *Bacillus* probiotic did significantly increase (P<0.05) in Feed conversion ratio (FCR).also Ziaei *et al*., (2006) found that treatment of *F. Indicus* with a commercial *Bacillus* probiotic did significantly increase (P<0.05) in average weight gain (g) Specific growth rate (%/d) Feed conversion ratio (FCR) particularly had received the probiotic both hatchery and farming stages. Nevertheless, similar results in some marine organisms as study conducted by (14) particularly had received the probiotic only during the farming stages found that did not significantly increase (P<0.05) in average weight gain (g) Specific growth rate (%/d) Feed conversion ratio (FCR). Also (32) found that treatment of *P. vannamei* with a commercial probiotic did not significantly increase (P<0.05) in Feed conversion ratio (FCR).

We observed daily feed intake and daily protein intake were decreased in all treatments whereas daily growth rate, daily weight gain and total production for postlarvae shrimp were increased compared with control these may be used as evidence or indicator of activity of digestive enzyme as indicated by studies[14; 41; 42] also there are many studies were manifested That positive effect of probiotic on the growth parameters because gram positive bacteria particularly members of the genus *Bacillus*, do secrete a wide range of exoenzymes[40; 18]. When of analysis of results to use regression we found through using Ecomarine these results were agreement with the study which conducted by[39] that found A regression analysis indicated that shrimp fed Ecomarine added diet performed the highest growth rate (slope 1.8 g/2 weeks). The growth rate of the control shrimp was slope (1.51 g/2 weeks) and of the Ecomarine added in rearing water was 0.99 g/2 weeks.

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