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Effect of Probiotics on the Survival and Production of Black Tiger Shrimp *Penaeus monodon* (Fabricius)

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Abstract: In recent years the use of probiotic bacteria in aquaculture has tremendous scope and the study of the application of probiotics in aquaculture has a glorious future. In the present study the probiotics, Silver-Ps was applied in culture ponds of *Penaus monodon* and was compared with control ponds (without probiotics). The salinity, pH, dissolved oxygen and temperature did not show significant difference between control and probiotics treated ponds. Average body weight, total production and survival rate of the ponds applied with probiotics were higher than that of control ponds. However feed conversion ratio was lower for the probiotics treated ponds than the control ponds. The shrimps in control ponds were seriously affected by bacterial infection; gill soaking and tail rot diseases. However, those problems are not encountered in probiotics applied ponds. The general conclusion obtained from the present study is that the probiotics plays a vital role in growth, survival and production of the shrimps by maintaining good water qnality parameters through out the culture period.

Key words: Probiotics, *Penaeus monodon*, silver-ps, vibriosis, tail rot, gill soaking

INTRODUCTION

In recent times, shrimp culture all over the world has been frequently affected by viral and bacterial diseases inflicting huge loss (Karuna Sagar *et al*., 1994). Pathogenic microorganisms implicated in these outbreaks were viruses, bacteria, rickettsia, mycoplasma, algae, fungi and protozoan parasites. For preventing and controlling diseases, antibiotics, pesticides and other chemicals were used possibly creating antibiotic resistant bacteria, persistence of pesticides and other toxic chemicals in aquatic environment and creating human health hazards. Based on the previous research results on probiotics suggest that the use of probiotic bacteria in aquaculture has tremendous scope and the study of the application of probiotics in aquaculture have a glorious future (Moriarty, 1997; Chen *et al*., 1992). The role of probiotics bacteria in small culture is studied but commercial level is not that much reported especially in giant black tiger shrimp, *P. monodon*. Hence, the beneficial effect of probiotics on the commercial culture of Indian major candidate shrimp, *P. monodon* is very much need of the hour. Therefore, the present study was aimed to examine the effect of a probiotics; Silver-Ps on the shrimp *P. monodon* culture was studied.

MATERIALS AND METHODS

The study was carried out in Mahalakshmi aqua farms located at Thirunagari, south east coast of India. Six ponds were selected for the present study and each pond was of 1.2 h area. For convenient the ponds were divided into two groups viz., control (without probiotics) and treated ponds (with probiotics) and each group had three ponds.

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Table 1: Amount of probiotics in different days of culture

Days of culture	Control pond (L ha^{-1})	Treated pond (L ha^{-1})
15	0	10
30	0	10
45	0	10
60	0	5
75	0	5
90	0	5
95	0	7
100	0	7
105	0	7
109	0	7

Healthy *P. monodon* seeds were purchased from a commercial hatchery (Tropical Marine). The seeds were stocked at a density of 8.3 m^{-2}. Before stocking, the seeds were acclimatized to the pond environmental conditions. The seed bags were allowed to float on the water surface in each pond for 30 min in order to adjust the temperature. The bags were opened and the pond water was introduced slowly by sprinkling in to the bags for 60 min to equalize with pond water quality. Subsequently, the bags were dragged to different parts of the pond and seeds were released slowly.

The shrimps were fed with CP feed. The feeding schedule was based on the feed chart given by the company. Blind feeding was done for first 25 days. Later the feeding was adjusted based on the check tray observation and sampling. Four check trays were installed in each pond for monitoring the animal health and feed intake. The feed ration was divided into 4 times a day as 25, 20, 30 and 25% in the morning (6.00 am), noon (11.00 pm) evening (6.00 pm) and night (10.00 pm), respectively. The feed was broadcasted from the rope method by using floats. Water exchange was not recommended for the first 30 days. After that 15 cm of water was exchanged regularly in every week.

Three ponds were treated with probiotics (Silver-Ps Hallmark company product) and remaining three ponds were without probioitics. The amount of probiotics given in treatment ponds is displayed in Table 1. It was broadcasted through out the ponds during morning hours.

Sampling was done in the ponds fortnightly during early hours of the day with a cast net. Five hauls were made in each pond. The shrimps were caught per haul and their individual weights are recorded. Healthiness, survival rate and Average Body Weight (ABW) of the animals were estimated. The water quality parameters of the probiotics treated and control ponds were regularly monitored.

At the end of culture period (109 days), the shrimps in both control and probiotics treated ponds were harvested. A bag net was fitted on outlet canal with 20 nnmbers mesh of width 1 m and length of 4 m. The water level in the ponds was reduced from 2 m to 60 cm and then out let was opened and shrimp was caught and collected. To know the statistical significance, the data was analyzed by using student's t-test.

RESULTS

The results of water quality parameters are shown in Table 2. Maximnm salinity was reported (48 ppt) at the DOC (days of culture) of 6. However minimnm was recorded at the end of the culture (16 ppt). There was no significant difference between control and probiotics treated ponds. The pH was alkaline throughout the culture period and did not show any significant difference between control and probiotics treated ponds. Dissolved oxygen levels in both control and treated ponds were varied between 3.8-4.9 ppm. However it did not show significant difference between control and probiotics treated ponds. The temperature of both control and probiotics treated ponds were varied between 23-33°C and did not show significant difference. Transparency in control ponds was ranged between 33-38 cm and it was 25-44 cm in probiotics treated ponds. The control and probiotics treated ponds showed significant difference with reference to transparency (Table 3 and 4).

Table 2: Water quality parameters of probiotics treated and control ponds

	Salinity (ppt)		pH		Dissolved oxygen (ppm)		Temperature (°C)		Transparency (cm)	
DOC	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
1	42±0.2	42±0.1	8.7±1.2	8.7±0.3	4.5±1.2	4.5±1.2	32±1.2	32±1.2	35±1.2	25±1.2
6	48±0.1	47±0.3	8.7±0.3	8.6±1.2	4.3±0.3	4.4±0.3	33±0.3	32±1.2	35±0.3	28±0.3
11	38±0.3	39±0.3	8.6±0.3	8.7±0.3	4.4±0.3	4.3±0.3	31±0.3	31±0.3	36±0.3	25±0.3
16	38±0.1	39±0.3	8.6±0.1	8.6±0.3	4.0±0.3	4.0±0.1	30±0.1	31±0.3	33±0.1	44±0.3
21	40±0.1	41±0.3	8.6±0.3	8.7±0.1	4.2±0.1	4.0±0.3	31±0.3	30±0.3	38±0.3	36±0.3
26	41±1.2	40±0.3	8.5±0.1	8.6±0.3	4.3±0.3	4.2±0.1	32±0.1	32±0.3	33±0.1	32±0.3
31	39±1.2	39±0.1	8.6±0.3	8.6±0.3	4.6±0.1	4.4±0.3	31±0.3	30±0.1	35±0.3	25±0.3
36	40±1.2	41±0.3	8.7±1.2	8.6±0.3	4.9±0.3	4.8±0.1	32±0.3	32±0.3	36±1.2	25±1.2
41	43±0.1	42±1.2	8.6±0.3	8.6±0.3	4.8±0.3	4.7±0.3	33±0.3	32±0.3	33±0.3	44±0.3
46	38±1.2	39±0.3	8.6±0.1	8.7±0.3	4.7±0.1	4.6±0.3	31±0.1	32±0.3	38±0.3	36±1.2
51	38±0.3	37±1.2	8.7±0.3	8.6±0.1	3.8±0.3	4.3±0.1	30±0.3	31±0.1	33±0.1	32±0.3
56	36±0.1	38±0.3	8.6±0.3	8.5±0.3	4.5±0.3	3.8±0.3	28±0.3	27±1.2	35±1.2	25±1.2
61	31±1.2	35±1.2	8.5±0.1	8.8±0.1	3.9±1.2	3.8±1.2	26±0.3	27±0.3	35±0.3	28±0.3
66	30±0.3	32±0.3	8.4±1.2	8.7±0.3	4.3±0.3	4.4±0.3	25±0.3	24±0.1	36±0.1	25±0.1
71	23±0.1	31±0.3	8.6±0.3	8.6±0.1	4.4±0.3	4.3±0.1	24±0.3	25±0.3	33±0.3	44±0.3
76	24±0.3	24±0.3	8.5±0.1	8.5±0.3	4.0±1.2	4.0±0.3	24±0.3	25±1.2	38±1.2	36±0.3
81	25±1.2	25±0.1	8.5±0.3	8.6±0.3	4.2±0.3	4.0±0.3	25±0.1	25±0.3	33±0.3	32±0.1
86	24±0.3	25±0.3	8.6±1.2	8.6±1.2	4.3±1.2	4.2±1.2	26±0.3	25±0.3	35±1.2	25±0.3
91	15±0.1	17±1.2	8.4±0.3	8.8±0.3	4.6±0.1	4.4±0.3	23±0.3	24±1.2	35±0.3	28±1.2
96	17±0.3	16±0.3	8.5±0.1	8.9±1.2	4.9±0.3	4.8±1.2	24±0.3	24±0.3	36±1.2	25±0.3
101	18±0.3	17±1.2	8.6±0.3	8.9±0.3	4.8±0.3	4.7±1.2	25±0.3	25±1.2	33±0.3	44±1.2
109	16±1.2	16±0.3	8.7±0.3	8.8±0.3	4.7±0.3	4.6±0.3	24±0.3	25±0.3	38±0.3	36±0.3

Table 3: Results of 't' test for the water quality parameters of control and treated ponds

Parameters	Control vs treated
Salinity	-2.84707
pH	1.08560
Dissolved oxygen	-2.34080
Temperature	1.08600
Transparency	3.24820*

*: Significant at p<0.001 level

Table 4: Average body weight of control and probiotics treated ponds

	ABW	
DOC	Control	Treated
64	9.50±0.3	10.99±0.3
81	12.56±1.2	15.08±1.2
85	14.28±0.3	17.04±0.1
92	15.75±0.1	18.49±0.1
90	18.00±0.3	21.48±0.3
109	19.95±0.1	23.32±1.2

The average body weight of the shrimps is significantly higher in the ponds treated with probiotics than that of control ponds. It increases when the culture period increases and this increment is higher in probiotics treated ponds rather than control ponds (Table 4).

Total production (956.700 kg) of the shrimps was significantly higher in the probiotics treated ponds than that of control ponds (593.900 kg). Maximum survival (89.2%) was recorded in the ponds treated with probiotics. However, it was comparatively minimum (65.9%) in control ponds. The difference was statistically significant. The shrimps in treated ponds were consumed more amount of feed (1152.520 kg) than in control ponds (833.050 kg). Food Conversion Ratio (FCR) was lower (1: 1.2) for the probiotics treated ponds than the control ponds (1:1.4) (Table 5 and 6).

Table 5: Total production, survival rate and FCR of control and probiotics treated ponds

Particulars	Control	Treated
Total production (kg)	593.900±0.1	956.700±0.1
Survival rate (%)	65.900±0.1	89.200±0.1
Feed intake (kg)	833.050±0.1	1152.520±0.1
FCR	1 : 1.400±0.1	1 : 1.200±0.1

Table 6: Results of t-test for average body weight, total production and survival of control and probiotics treated ponds

Particulars	Control vs treated
Average body weight	7.812*
Total production	403.300*
Survival	44.220*

*: Significant at p<0.001 level

DISCUSSION

The present study was undertaken to ascertain the efficiency of probiotics (Silver-Ps) on the average body weight, total production and survival of the most important cultivable shrimp species, *P. monodon* in addition to its influence on important water quality parameters. Important water quality parameters monitored during the present study were, salinity, pH, dissolved oxygen and temperature.

The quality of water during the culture period will deteriorate mainly due to the accumulation of metabolic wastes of living organisms, decomposition of unutilized feed and decay of biotic materials. Generally organisms are in a state of balance between potential disease causing microorganisms and their environment. Change in this equilibrium through way of impairment in water quality parameters can influence survival of organisms as they become vulnerable to disease due to stress, so also growth. Efficient removal of imbalances, which cause impairment in water quality, is difficult. However addition of some commercial preparations as probiotics is reported to effectively deal with these substances and that way helpful in maintaining water quality parameters thereby improving growth rate, weight gain and survival rate with an attractive FCR in farmed organisms (Sissons, 1989).

In the present study, the water quality parameters of the ponds, which are applied with microbial supplement through probiotics, was good because of the various roles played by the microbes. Improved water quality has especially been associated with *Bacillus* sp. The rationale is that gram-positive bacteria are better converters of organic matter back to CO_2 than gram-negative bacteria. During the production cycle, high levels of gram-positive bacteria can minimize the buildup of dissolved and particulate organic carbon. A similar observation was found in the present study. The pond that was treated with the Silver-Ps was abundant with *Bacillus* sp. was showing a low level of Ammonia, which was converted into nitrate through nitrite. Thus probiotic application lead to the nutrient enrichment through the way of microorganism, releasing nutrients by decomposing the waste material that accumulate in pond bottom as left-over feed and die-off. This nutrient enrichment leads to increased phytoplankton production and photosynthetic activity.

Salinity is the most important factor influencing many functional responses of organisms as metabolism, growth, migration, osmotic behavior, reproduction etc. At high salinity the shrimp will grow slowly but they are healthy and resistance to diseases. If the salinity is low the shell will be weak and prone to diseases. So optimum salinity is important for normal growth. Muthu (1980) and Karthikeyan (1994) recommended a salinity range of 10-35 ppt was ideal for *P. monodon* culture. While Chanratchkool *et al*. (1994) maintained the salinity of 10-30 ppt. Chen (1980) opined that salinity ranges of 15-20 ppt are optimal for culture of *P. monodon*. There are few reports (Shivappa and Hambry, 1997; Ramakrishna Reddy, 2000; Collins and Russel, 2003), which stated that *P. monodon* adapted quite well in freshwater conditions also because of its wide range of salinity tolerance. In the present study the salinity was ranged between 16-48 ppt in both control and probiotics treated ponds. But there was no significant difference between control and probiotics treated ponds in terms of salinity.

pH of the culture is having an important say on the metabolism and other physiological processes of organisms. It is a very good indicator of presence of metabolites, photosynthetic activity and fertility of culture medium. It changes with accumulation of residual feed, dead algae and excreta under farming conditions. It is at its maximum when photosynthesis is maximum (vigorous) and decreases when there is none. High pH means pond water is too fertile and therefore there is a possibility of planktonic bloom. Moreover the toxicity of ammonia is pH linked. In the optimum range of pH, ammonia will not cause much problem. Low pH means water is infertile, planktonic growth is low and less oxygen is produced from photosynthesis. Toxicity of nitrite and hydrogen sulphide is increased in low pH. The optimum range of pH 6.8 to 8.7 was maintained for maximum growth and production of penaid species (Ramanathan *et al*., 2005). Reddy (2000) was recommended pH of 7.5 to 8.5 for *P. monodon* culture. This range is considered good for shrimps because certain salts like bicarbonate are to be present essentially in the culture medium for growth, reproduction and other physiological activity. In the present study the pH was alkaline throughout the culture period and did not show any significant difference between control and probiotics treated ponds.

Oxygen dissolved in the culture medium is an important factor not only for the respiration of aquatic organisms but also to maintain favorable chemical and hygienic enviroument of the water body. It controls many oxidation reactions and maintains aerobic conditions in water. When oxygen level is very low and anaerobic conditions exist, nitrate is reduced by denitrifies into ammonia, which will be toxic. This also increases the pH. Low-level of oxygen tension hampers metabolic performances in shrimp and can reduce growth and moulting and cause mortality (Molluae, 2001). Oxygen level in the culture medium can be maintained in the desired range by aeration. Continuous aeration was provided during the present study and therefore the oxygen level did not vary between the control and experimental tanks and was in the range of 3.8-4.9 ppm.

Water temperature is probably the most important enviroumental variables in shrimp culture, because it directly affects metabolism, oxygen consumption, growth, moulting and survival. In general, a sudden change of temperature affects the shrimp immune system. The optimum range of temperature for the black tiger shrimp is between 26 to 30°C (Ramanathan *et al*., 2005). The temperature in the present study was 23-33°C. There was no marked difference in temperature between control and probiotics treated ponds.

The transparency is maiuly depends on the presence of phytoplankton. The secchi disc reading should be between 30-40 cm (Anonymous, 2006). The optimum range of secchi disc reading is between 30 to 60 cm to the juvenile stage and between 25 to 40 cm to the sub adult and final stage. The transparency of the present study is 25-44 cm. Reddy (2000) also observed similar transparencies (25-50 cm) for his study. The reading less than 30 cm mean that the phytoplankton density is high. If it is more than 40 cm indicates, low population of phytoplankton. In the present study such high values of transparency was reported in control ponds.

Feed is one of the essential inputs in shrimp production and increase profits. Feed management is highly subjective, as feed consumption cannot be directly observed. In the present study CP feeds was used for all ponds and the amount was followed as per feed chat. Maximum amount of feed (1152.520 kg) was consumed by the shrimps in probiotics treated ponds and was less in control ponds (833.050 kg). This indirectly supports less food conversion ratio in the probiotics treated ponds than in control ponds. Average Indian cultured food conversion ratios were varying between 1.5 to 1.75 (Paul Raj, 1999). Chekait (1995) observed the food conversion ranges were varying from 1.50 to 1.55 when microencapsulated diets are used. Saha *et al*. (1999) observed that the food conversion ratios of 1.31 to 1.58 in low saline ponds and 1.35 and 1.68 in high saline ponds. Reddy (2000) observed FCR of 1.58 for his study. In the present study FCR of both control and treated ponds were 1:1.2, 1:1.4, respectively.

Periodic sampling is very vital for successful shrimp culture. It is recommended to do weekly or fortnightly sampling to check the health condition as well as to estimate the growth of shrimps. Sampling also helps to know the average weight and this would help in estimating the total biomass in the pond for better-feed management. Average growth rate of shrimps depends maiuly on pond water quality and effective management of feeding. It is observed that average growth rate of shrimps in the present study is rapidly increasing after DOC 64 in all ponds due to the accurate feed manipulation by sampling. However, the average body weight was high in probiotics treated ponds rather than control, ponds.

In the present study higher survival (89.2%) was recorded in the probiotics treated ponds and the lower survival (65.9%) was in control ponds. It was achieved due to the application of probiotics and required stocking density of 4,000 ha^{-1}. Krantz and Norris (1975) stated that survival rates of 60 to 80% are to be expected for *P. monodon* under suitable rearing conditions. Average survival of 70-80% is quite possible if the idle conditions are maintained for *P. monodon* (Reddy, 2000). The total production of the present study was observed to be maximum in probiotics treated ponds (956.700 kg) than control ponds (593.900 kg).

In the present study bacterial infection was observed in the shrimps of control ponds. Shrimps were always under stress and eventually weak. Some shrimps were seriously affected by gill soaking. This is due to dark brown colour of the water. Due course of time the colour of the control ponds were changed into black. The shrimps cultured under these conditions were dirty and tail rot was appeared. The above said problems were not encountered in the shrimps in probiotics treated ponds. Since the probiotics maintain good water quality as evidenced by high survival and production.

Shrimp aquaculture production in much of the world is depressed by disease, particularly caused by luminous *Vibrio* and/or viruses. The probiotics treated ponds in the present study had either a very low abundance or a complete absence of luminous and very good survival was achieved. Since the shrimps in the control pond were dominated with *Vibrio* sp., which caused Vibriosis, can be attributed as the reason for low survival in the control ponds when compared with probiotics treated ponds. The occurrence of Vibriosis in the control pond was concluded by presence of luminescence in the nighttime and occurrence of dead animals in the check tray. The general conclusion obtained from the present study is that the probiotics plays a vital role in growth, survival and production of the shrimps by maintaining good water quality parameters throughout the culture period.

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