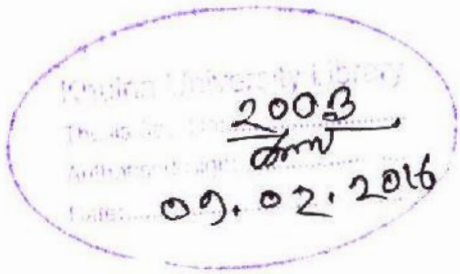
EFFECT OF PROBIOTICS ON THE GROWTH AND SURVIVAL OF BLACK TIGER SHRIMP (*Penaeus monodon*)



A Thesis

By

Samar Ghosh

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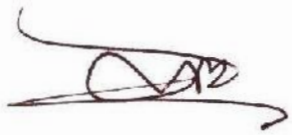

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Declaration

EFFECT OF PROBIOTICS ON THE GROWTH AND SURVIVAL OF BLACK TIGER SHRIMP (*Penaeus monodon)*

The results presented in this thesis with the above mentioned title are entirely of the candidate's own investigations and no part of the results has been accepted for any degree nor is it being concurrently submitted for any degree.

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TO

MY BELOVED PARENTS

Acknowledgement

All praises are due to the Almighty God, the supreme Authority of the universe, who empowers me to complete the research work and the thesis for the degree of Master of Science (M.S.) in Fisheries.

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Samar Ghosh

Abstract

The present investigation was carried out to evaluate the effects of commercial probiotics on the production of black tiger shrimp (*Penaeus monodon*) in comercial culture systems in Satkhira district of Bangladesh. With considering the principles of probiotics' application in aquaculture, the study was designed as to primarily compare the growth and survival of shrimp between with-probiotic and without probiotic (control) culture-systems. Super PS as soil probiotics, Super Biotic and PH Fixer as water probiotics and Zymetin as nutrient probiotics supplied by CP Aquaculture Private Limited, India were used in culture ponds of 0.4 ha with stocking density of 10 PLs/m^2 for 120 days. Feeding with CP feed and each specific probiotics were administrated in line with the guidelines of CP Company. There were no significant differences among water parameters pH, dissolve oxygen between control and probiotic-treated ponds. The average body weight and total production were observed to be higher in probiotics-treated ponds than in control ponds. 75% survival was found in probiotics-treated ponds whereas 65% in control. Lower (1.3) FCR was reported for the probiotics-treated ponds compared to control ponds (FCR value, 1.7). The findings of the present work imply that probiotics could be used in shrimp farming systems, and thus can play a significant role in sustainable aquaculture practice.

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List of Abbreviations

ABW-Average Body Weight

ADG- Average Daily Gain

CP- Cheron Phokphand

DO- Dissolve Oxygen

DoC- Day of Culture

FCR- Feed Conversion Ratio

CHAPTER - 1

INTRODUCTION

1. Introduction

Modern fish culture means improvement of culture practices through adopting different measures such as optimum stocking density, regular feeding, maintenance the physio-chemical water parameters, disease prevention and various control measures. In addition use of probiotics in culture system recently enhances production manifolds. However, these cultural techniques are not properly followed in this country. As a result production of fish and shrimp are very low compared to others developing countries in the world. It is perhaps the right time to think to how the fish production can be increased at minimum time and cost.

In recent times, shrimp culture all over the world has been frequently affected by viral and bacterial diseases inflicting huge economic loss (Karuna Sagar et al., 1994). Pathogenic microorganisms responsible in outbreaks are viruses, bacteria, rickettsia, mycoplasm, algae, fungi and protozoan parasites. For preventing and controlling diseases, antibiotics, pesticides and other chemicals was used which possible creating antibiotic resistant bacteria, persistent of pesticides and other toxic chemicals in aquatic environment resulted human health hazards. Based on the previous results on probiotics suggest that the use of probiotic bacteria in aquaculture have a potential future for combating diseases (Moriaty, 1997; Chen et al., 1992). The role of probiotic bacteria in small scale culture is studied but commercial level is not that much reported especially in shrimp, *Penaeus monodon.* Hence, the bacterial effect of probiotics on the growth and survival of *p. monodon* in our country is a new era of shrimp culture production. Therefore, the present study was aimed to examine the effect of probiotics (commercially available) on the shrimp *P. monodon* culture.

Objectives of the study

- To know the effect of probiotics on shrimp culture management i.e. growth, survival and production
- To know the effect of probiotics on water quality parameters in the culture system

CHAPTER - 2

MATERIALS & METHODS

2. Materials and Methods

2.1 Study area and study period

The study was conducted in Mahi Fish Culture farm, Noarpara, Debhata, Satkhira. The study was conducted during the month May to September, 2009 with a culture period of 120 days.

2.2 Experimental design

Six ponds were selected for the present study and each pond was of 0.4 h area. The ponds were divided into two groups, viz., control (without probiotics) and treated ponds (with probiotics) and each group contain three ponds.

2.3 Process of data collection

Total culture system was supervised by own observation. All the required data were collected by direct observation. Water quality parameters (P^H, DO, Alkalinity) were collected in regular basis with weekly basis. Sampling was done in every week throughout the culture period.

2.4 Probiotic culture system in different stocking densities

Continuous stocking was not done; PL was stocked in a definite time. PL were collected from hatchery source.

PROBIOTIC FARMING SYSTEM

A. WATER PREPARATION

1. SUPER PS at 10-20 L/ha /Time for wet pond preparation
2. Eradication of virus carriers by using CHLORINE (30 ppm)
3. MOLASES, RICE BRAN and YEAST (2:1: 0.01) fermentation at 20-25 Kg/ha/day for 1 week to enhance p^H stability
4. P^H FIXER at 6 Kg/ha/for every 4 days for two weeks for stable water p^H
5. DOLOMITE at 50-60 Kg/ha used in 2 or 3 doses depending on P^H

B. POST LARVE (PL) CULTURE

1. SUPER BIOTIC at 3 Kg/ha/once a week for 6 weeks to reduce pathogenic bacteria
2. SUPER PS at 10 L/ha/once a week for 16 weeks to improve pond bottom soil
3. ZYMETIN at 10 g/Kg feed /one meal daily for 16 weeks to prevent luminescense bacteria in the gut and to avoid white feaces
4. MUTAGEN at 10 g/Kg feed /one meal daily to help for increasing feed intake
5. VITAMIN C-150 at 5 g/Kg feed/one meal daily for better growth

C. HARVEST

2.5Data analysis

2.5.1 Growth monitoring

During morning sampling is done for growth monitoring. Shrimp weight was taken by using balance and then divided it by shrimp number.

$$\text{Body Weight} = \frac{\text{Weight of shrimp}}{\text{Number of shrimp}}$$

2.5.2 Feed Conversion Ratio (FCR)

$$\text{FCR} = \frac{\text{Total feed used in the pond}}{\text{Total biomass of that pond}}$$

2.5.3 Survival rate calculation

$$\% \text{ Survival rate} = \frac{\text{Number of Shrimp in Pond at Present}}{\text{Number of Shrimp in Initial Stocking}} \times 100$$

2.6 Sources of PL

Healthy P. monodon seeds were purchased from commercial hatchery.

2.7 Acclimatization

Before stocking, the seeds were acclimatized to the pond environment conditions. The seed bags were allowed to float on the water surface in each pond for 15 min to adjust the temperature. The seed bags were opened and the pond water was introduced slowly by sprinkling into the bags for 60 minutes to equalize with pond water quality. Subsequently, the bags were dragged to different parts of the ponds and seeds were released slowly.

2.8 Stocking in Grow-out pond

The PL were stocked at a density of 10 pcs/m2. The total stocking situation was given below;

Table 1: Stocking of PL in different ponds

Serial no.	Control ponds			Treated ponds		
	Area (ha)	Stocking Density	Stocking nos	Area (ha)	Stocking Density	Stocking nos
1	0.4	10 pcs/m2	40,000	0.4	10pcs/m2	40,000
2	0.4	10pcs/m2	40,000	0.4	10pcs/m2	40,000
3	0.4	10pcs/m2	40,000	0.4	10pcs/m2	40,000

2.9 Feeding in Grow-out ponds

The shrimps were fed with commercial feed CP. The feeding schedule was based on the feed chart given by the company. Blind feeding was done for the first 30 days. Later the feeding rate was adjusted based on the check tray observation and sampling. Four check trays were installed in each pond for monitoring the shrimp health and feed intake. After 2 hours of feeding, check tray were monitored. The fed ratio was divided into four times a day as 25%, 20%, 30% and 25% in the morning (6.00 am), noon (11.00 am), afternoon

(5.00 pm) and night (10.00 pm), respectively. The feed was broadcasted from the rope methods by using floats.

2.10 Application of probiotics

Three ponds were treated with soil probiotic (Super PS, CP company product), water probiotics (Super Biotic & PH Fixer, CP company product) and nutrient probiotic (Zymetin, CP company product) and other three ponds were without probiotics. The total amount of probiotics given in following tables and its application were shown in materials section.

Table 2: Total Amount of probiotics in different ponds

Product name	Use in control ponds	Use in treated ponds
Super PS	0	80 L
PH Fixer	0	18 Kg
Super Biotic	0	18 Kg
Zymetin	0	14 Kg

2.11 Sampling

Periodic sampling is very vital for successful shrimp culture. Sampling was done in the ponds fortnightly during early hours of the day with a cast net. It helps to know the health condition of the shrimp as well as the average body weight of the shrimp. It also helps to know the total biomass of the pond for the better feed management of the pond. Average growth rate of shrimps depends mainly on pond water quality and effective management of feeding. However, the average body weight was high in probiotic treated ponds rather than control ponds.

CHAPTER - 3

RESULTS

3. RESULTS

3.1 Water Quality Parameters

The quality of water during the culture period will deteriorate mainly due to accumulation of metabolic wastes of living organisms, decomposition of unutilized feed and decay of biotic materials. 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, important water quality parameters salinity, P^H and Dissolve Oxygen were monitored.

3.1.1 pH in both types of ponds

P^H of the culture is having an important say on the metabolism and other physioligal processes of organism. The optimum range of pH 6.8 to 8.7 was maintained for maximum growth and production of penaid species (Ramanathan *et al.*, 2005). In the present study, pH throughout the culture period did not show any significant differences. This pH ranges were good for growth and survival.

Table 3: pH of the ponds

DoC	Treated pond	Control pond
1	8.2 ±0.5	8.3 ±0.6
7	8.3 ±0.4	8.4 ±0.7
14	8.3 ±0.4	8.4 ±0.8
21	8.4 ±0.3	8.6 ±0.5
28	8.4 ±0.2	8.6 ±0.4
35	8.4 ±0.3	8.7 ±0.4
42	8.5 ±0.4	8.6 ±0.3
49	8.6 ±0.1	8.6 ±0.7
56	8.5 ±0.6	8.8 ±1.1
63	8.4 ±0.3	8.8 ±0.9
70	8.5 ±0.8	8.9 ±0.5
77	8.4 ±0.7	8.9 ±0.7
84	8.2 ±0.9	8.5 ±0.9
91	8.3 ±0.7	8.6 ±0.3
98	8.4 ±0.2	8.5 ±0.5
105	8.4 ±0.4	8.6 ±0.8
112	8.5 ±0.5	8.7 ±0.5
119	8.6 ±0.3	8.8 ±0.1

3.1.2 Dissolve Oxygen in Different ponds

Dissolve oxygen in the culture medium is an important factor not only for the respiration of aquatic organisms but also maintain favourable chemical and hygienic environment of the water body. Oxygen level in the culture medium can be maintained in the desired range by aeration. Continuous aeration was provided during the present study therefore oxygen level both in control and treated were in the range of 3.5 to 4.5 ppm.

Table 4: DO in treated and control ponds

DoC	Treated pond	Control pond
1	4.2 ±0.5	4.2 ±0.3
7	4.3 ±0.4	4.2 ±0.2
14	4.3 ±0.4	4.1 ±0.3
21	4.4 ±0.3	4.2 ±0.1
28	4.4 ±0.2	4.1 ±0.2
35	4.4 ±0.3	4.0 ±0.3
42	4.5 ±0.4	3.8 ±0.2
49	4.6 ±0.1	4.0 ±0.2
56	4.2 ±0.6	4.0 ±0.3
63	4.3 ±0.3	3.5 ±0.1
70	4.4 ±0.8	3.5 ±0.1
77	4.0 ±0.7	4.0 ±0.2
84	4.2 ±0.9	3.6 ±0.2
91	4.5 ±0.7	3.5 ±0.3
98	4.4 ±0.2	3.5 ±0.2
105	4.4 ±0.4	3.0 ±0.2
112	4.5 ±0.5	3.5 ±0.1
119	4.0 ±0.3	3.5 ±0.3

3.2 Average Body Weight of two different ponds

The average body weight of the shrimp was significantly higher in the ponds treated with probiotics than that of control ponds.

Table 5: Average Body Weight in control and treated ponds

DoC	Control pond	Treated pond
35	5g±0.1	4g±0.2
42	8g±0.1	6g±0.1
49	10g±0.2	7g±0.2
56	12g±0.3	9g±0.2
63	14g±0.1	11g±0.1
70	16g±0.3	12g±0.1
77	18g±0.2	14g±0.3
84	20g±0.1	16g±0.2
91	23g±0.3	17g±0.2
98	26g±0.2	19g±0.1
105	29g±0.2	19g±0.1
112	32g±0.4	20g±0.2
119	35g±0.2	22g±0.2

3.3 Total Production, Survival rate and FCR of control and treated ponds

Total production (1050 Kg) of the shrimp was significantly higher in the probiotics treated ponds than that of control ponds (572.5 Kg). Maximum survival (75%) was recorded in the ponds treated with probiotics. However, it was comparatively minimum (65%) in control ponds. The shrimp in treated ponds were consumed more amount of feed (1365.5 Kg) than in control ponds (973.25 Kg). Food Conversion Ratio (FCR) was lower (1.3) for the probiotics treated ponds than the control ponds (1.7).

Table 6: Total Production, Survival rate and FCR of control and treated ponds

Particulars	Control Pond	Treated Pond
Total Production (Kg)	572.5	1050
Survival Rate (%)	65	75
Feed Intake (Kg)	973.25	1365.50
FCR	1.7	1.3

CHAPTER - 4

DISCUSSION

4. DISCUSSION

The present study was undertaken to ascertain the efficiency of probiotics on the growth, production and survival of the most important cultivable shrimp species, *P. monodon* as well as the pond water quality through out the culture period.

The quality of water during culture period will deteriorate mainly due to accumulation of metabolic wastes of living organisms, decomposition of unutilized feed and decay of biotic materials. 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. 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 this present study, the water quality parameters of the ponds, which are applied with microbial supplement through probiotics, was good because of various roles played by the microbes. Improved water quality has specially associated with *Bacillus sp.* (Sissons, 1989).

Salinity is the most important factor influencing many functional responses of organism. At high salinity the shrimp will grow slowly but they are healthy and resistance to disease.

pH of the culture is having an important say on the metabolism and other physiological processes of organism. 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. 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 did not show significant differences between control and treated ponds.

Dissolve oxygen in the culture medium is an important factor not only for the respiration of aquatic organisms but also maintain favorable chemical and hygienic environment of the water body. It controls many oxidation reactions and maintain aerobic condition in water. 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 therefore oxygen level both in control and treated were in the range of 3.5 to 4.5 ppm.

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 chart. Maximum amount of feed (1365.5 Kg) was consumed by the shrimps in probiotics treated ponds and was less in control ponds (973.25 Kg).This indirectly supports less food conversion ratio in the probiotic treated ponds than in control ponds. In semi-intensive culture system feed conversion ratios were 1.4 to 1.7 (Paul Raj, 1995). In the present study FCR of both treated and control ponds were 1.3 and 1.7 respectively.

Periodic sampling is very vital for successful shrimp culture. It is recommended to do weekly to check the health condition as well as to estimate the growth of shrimps. It also helps to know the total biomass of the pond for the better feed management of the pond. Average growth rate of shrimps depends mainly on pond water quality and effective management of feeding. However, the average body weight was high in probiotic treated ponds rather than control ponds.

In the present study higher survival (75%) was found in the probiotics treated ponds and the lower survival (65%) was in control ponds. It was achieved due to the application of probiotics. Krantz and Norris (1975) stated that survival rates of 60 to 80% are to be expected for *P. monodon* under suitable rearing condition. The total production of the present study was observed to be maximum in probiotics treated ponds (1050 Kg) than control ponds (572.5 Kg).

Bacterial infection was observed in the shrimps of control ponds. During the culture period some shrimps were seriously affected by gill soaking. This is due to dark brown color of the water. The above problems were not found in probiotics treated ponds.

Shrimp aquaculture production in many corners of the world is depressed by disease, particularly caused by luminous *Vibrio* and virus. The probiotics treated ponds had either a very low abundance or a complete absence of luminous bacteria and hence good survival was achieved. The general conclusion obtained from the present study is that probiotics plays a vital role in growth. survival and production of the shrimps by maintaining good water quality parameters throughout the culture period.

Conclusions

Probiotics plays significant role in growth, survival and production of the shrimps by maintaining good water quality parameters through out the culture period. The salinity, pH, Dissolve Oxygen did not show significant differences between control and treated ponds. Average Body Weight (ADG), total production and survival rate were higher in probiotics treated ponds than control ponds. However, Feed Conversion Ratio (FCR) was lower for the probiotics treated ponds than control ponds.

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APPENDIX

Appendix

Probiotic Product Information

SUPER BIOTIC (WATER PROBIOTIC FOR VIBRIOSIS CONTROL)

Out break of Vibriosis mainly by luminescence bacteria is more in summer due to high temperature and high salinities. CP Group scientists in Thailand have done intensive research and found out special strain of Bacillus to have great inhibitory abilities against luminescent vibrio and have named it as SUPER BIOTIC.

COMPOSITION:

Baccillus sp. 10 cfu /g product. This strain of Bacillus was scientifically proven to have great inhibitory abilities luminescent Vibrio.

DIRECTION FOR USE:

Condition	Vibrio count	Doses
For prevention	Less than 100 cfu/ml	3kg/ha/week
At high salinities & high temperature	100-500 cfu/ml	6kg /ha/week
Vibrio out break & weak shrimp	More than 500 cfu/ml	12kg/ha/week

Mix 1kg of Super Biotic with 4litre of clean water and leave the mixture for 6-12 hours prior to use. Broadcast all over the pond or in front of the paddlewheel during the morning time.

P^H FIXER (WATER PROBIOTIC)

P^H Fixer is the best remedies in maintaining water quality and P^H stability of the pond water. This product has *Bacillus* Strain that inhibit the multiplication of pathogenic vibrio. It helps to restore stable microbial and balance the ecosystem after water treatment, thus maintaining a natural environment suitable for PL stocking.

Composition

New strain of *Bacillus sp.* 10^9 CFU/ gm, microbial media and buffer mixture.

Direction for Use:

After water treatments apply 4-6 Kg of product per hector once every 4 days for 2 weeks before stocking. Mix clean pond water (2 Kg fixer per 20 liters of pond water) and stir for 5-10 minutes and broadcast through the pond or in front of paddle wheel aerator during noon.

SUPER PS (SOIL PROBIOTIC)

Super PS is a beneficial microbial culture aimed to improve shrimp growth and pond bottom condition. It is composed mainly on *Rhodobacter* and *Rhodococcus* that is designed to biodegrade soluble organic pollutants of the pond.

Composition

Rhodobacter sp and *Rhodococcuss sp* in 10^9 CFU/ml

Direction for Use

Apply 4 lit/acr at 9 am to11 am in a sunny day. Mix super ps with sand and if P^H below 7 then mix super ps with Dolomite until the sand or Dolo remove all the liquid and spread it through the feeding line.

ZYMETIN (FEED PROBIOTIC FOR BALANCED GUT MICRO FLORA)

Zymetin is beneficial bacteria that aim to improve the health of the shrimp. It is usually mixed with feed to inhibit the growth of pathogenic bacteria in the gut. It also improve the appetite of the shrimp to increase growth rate and reduce FCR.

Composition

1. *Streptococcus faecalis*
2. *Bacillus mesentericus*
3. *Clostridium butyricum*
4. Protease
5. Lipase
6. Beer Yeast

Dosage

Use Zymetin at 5 gm/ Kg feed, mix zymetin first with fresh water and then mixed properly with feed.