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Observation of probiotics effect on the growth, survival and production of giant freshwater prawn (*Macrobrachium rosenbergii*) in south-west part of Bangladesh

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Abstract

The study was conducted to observe the effect of probiotics on growth and survival of giant freshwater prawn, *Macrobrachium rosenbergii* from September to January 2018. There were 3 experimental groups viz (a) control or without probiotics treated prawn (T_1), (b) water probiotics (PRO-W) treated prawn (T_2), and (c) feed probiotics (Aqualact™) treated prawn (T_3). Stocking density was 3PL/m² for all and each was triplicated. During stocking, average body weight of juvenile prawn was 1.93 g. Feeds were applied at 10% body weight in 1st month and reduced to 7% at 2nd month, 5% at 3rd month onward till the end. The lowest and highest final weight of prawn was found in T_1(40.13±3.9 g) and T_3 (54.73±2.87g) respectively at the end. The SGR was found to be 2.02±1.51, 2.16±1.65 and 2.23±1.87 (%BW/day) in T_1, T_2 and T_3 respectively. The survival rate differs significantly and highest was found in in T_3 (93.86±1.42%). The average FCR (Feed Conversion Ration) was lowest in T_3 (1.13±0.15) and highest inT_1 (2.27±0.43). The net average production was found highest in T_3 (1090.27±60.65kg/ha). Water quality parameters were measured regularly and were within the culturable range. The production of probiotics treated ponds was always higher than without probiotics treated ponds. So, it can be concluded that using controlled probiotics can minimize the cost of production and hence increase the benefit of farmers.

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Introduction

Bangladesh is very much blessed with riverine giant prawn(*Macrobrachium rosenbergii*, locally called as golda) culture becauseof some suitable conditions like geographic position, vast amount of freshwater and brackish water,agro-climaticenvironment, culture technique and avail-ability of wild PL (Ahmed *et al.*, 2008a; Wahab *et al.*, 2012). This species of prawn has remarkable advantage, because of its omnivorous feeding habit, high growth rate, good amount of protein, tolerance of wide range of temperature (15-35°C) and resistance to diseases (Ling, 1969). The production level of prawn is low in Bangladesh compared to those other countries. With the increasing intensification and commercialization of aquaculture production, disease problems inevitably emerge. In recent decades, the abuse of antimicrobial drugs, pesticides, and disinfectants in aquaculture disease prevention and growth promotion has led to the evolution of resistant strains of bacteria as well as a question of safety (Esiobu *et al.*, 2002). Typically, farmers use chemicals and antibiotics in their prawn farming, and very recently they have been introduced to probiotics and employing them in their hatchery and grow-out ponds considering the demand for more disease-free, environment-friendly and sustainable aquaculture practice. There are increasing reports of research works on the application of probiotics in aquaculture though very few studies related to the usage of probiotics in prawn culture available in Bangladesh (Li *et al.*, 2006; Hai *et al.*,2009; Zhou *et al.*, 2009). The World Health Organization has defined probiotic bacteria as "live microorganisms which when administrated in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). Probiotic bacteria could producedigestive enzymesand essential growth nutrients such asvitamins and amino acids, which are benefit for enhancingthe best growth, also they could benefit to theirinvertebrate host by competitive exclusion against pathogens(Austin *et al.*, 1995; Gomez-Gil *et al.*, 2000) or byincreasing the host resistance and immunity (Uma *et al.*,1999) which are benefit to achieve the higher survivalrate and healthier animals.Appropriate uses of probiotic in aquaculture industrywere shown to improve intestinal microbial balance, andalso to improve feed absorption, thus leading to increasedgrowth rate (Parker, 1974; Fuller, 1989; Rengpipat *et al.*, 1998) and also reduced feed conversion ratio (FCR)during the cultural period (Wang, 2005). The present study was attempted to observe the effect of probioticson growth, survival and production of the freshwater prawn *M. rosenbergii*.

Methodology

Study area and time

The experiment was conducted for five months in in Sadar Upzilla under Bagerhat district from September 2017 to January 2018. The experiment was conducted with the giant freshwater prawn (*Macrobrachium rosenbergii*) together with some commercial probiotic bacteria.

Experimental design

To study the growth performance, survival rate and production potential of *Macrobrachium rosenbergii*, three treatments were selected following a control group, each with three replications. The control group was cultured without any probiotic treatment and was expressed as T_1.The first treatment group of prawn T_2 was cultured using only water probiotics (PRO-W)The second treatment groupof prawn T_3, was cultured using only, feed probiotics (Aqualact™).

Pond preparation

Unwanted aquatic vegetation and weed were manually cleaned before starting the experiment. Lime was applied at 2 kg/decimal (5 days after drying). After liming, the ponds were filled with adjacent deep tube well water. All ponds were fertilized initially with urea and triple super phosphate (TSP) at the rates of 300g and 200g per decimal respectively. TSP was dissolved in plastic buckets 6 hours before application, then urea and TSP were mixed together and applied into the ponds by spreading methods, equaling 70% of the pond surface area. After fertilization, the ponds were left 3 days to allow plankton development. The total area of the ponds was fenced with fine meshed nylon net.

PL nursing and stocking

The post larvae (PL) of prawn was collected from the local nursery of Bagerhat district. PL was reared in the nursing pond for 30 days with a stocking density of 22 PL/m². After nursing PL, itbecame juvenilewhich was stocked in grow-out ponds with astocking density of 3/m²while the average individual weight was1.93g.

Feeding trials

In this experiment supplementary feeds (Quality gold) was applied daily for better growth. Before feeding, the feed was analyzed to determine the proximate composition according to the standard procedures given by Association of Official Analytical Chemists (AOAC, 1980). The average percentage of protein, lipid, ash and moisture was 31.50±0.07, 6.79±0.02, 4.91±0.20 and 8.98±0.11% respectively in the feed. Feeds were applied at 10% body weight at 1st month (up to 30 days) and reduced to 7% at 2nd month, 5% at 3rd month and were continued till the end of the experiment. Feed was distributed evenly over the ponds surface twice daily at 07:00 A.M. and 6:00 P.M.

Composition and application of probiotics

Two commercial probiotics were used in the experiment. The trade name of probiotics Aqualact™ (feed probiotics) and PRO-W (water probiotics) provided by Biostadt India limited and Sanolife private limited respectively. The com-position and the mode of application were described on the label (pack) of the probiotics which were given below.

Aqualact™

Aqualact is a feed probioticmade with beneficial bacteria which is used to prevent the growth of pathogens. It also improves the appetite and increase function of digestion and assimilation for better growth and reduces FCR. It is composed mainly with *Bacillus licheniformis, Lactobacillus acidophilus, Bacillus subtilis, Lactobacillus sporogenes*, Protease, phytase, Amylase, Lipase, Cellulase, Beta-galactosidase and vitamin (B_1, B_6 and C). The application procedure of this probiotics was as follows; 10g Aqualact was per kg feed for first four days in a week.

PRO-W

PRO-W is an advanced probiotic specially formulated to promote rapid decomposition of waste material and help to control pathogenic bacteria in ponds under both aerobic and anaerobic conditions. It is also stabilizing the phytoplankton bloom and keeps water quality optimal and reduces ammonia and nitrate from the water. PRO-W can be used in both fresh and marine water.It is composed of specific mixture of strains of *Bacillus Spp*. Concentration of bacteria is minimum $5x10^{10}$cfu/g. The application procedure was, before liming the ponds apply 100-200g/ha. After filling the ponds apply 100-200g/ha/1m depth. During culture apply 100-200g/ha on a weekly and bi-weekly basis. To shorten the dry out period after harvest apply 500g/ha directly to the wet soil of an empty pond.

Measurement of water quality parameters

The important parameters of pond water such as temperature, P^H, dissolved oxygen, transparency, alkalinity and total hardness were inspected and monitored regularly basis. Water temperature was recorded with Centigrade Thermometer (YSI, model 58, USA). Dissolved Oxygen (DO) and pH were recorded by digital DO (HACH Sension6) & pH meter (HACH Sension PH1). Alkalinity and hardness were recorded by Hach kit (FF-1A) and transparency was recorded by secci disk. Sampling was done monthly.

Data collection

The growth of prawn was monitored monthly from each pond through random sampling method. The sampling was done with the help of a cast net for the determination of their total body weight and length. Growth of prawn in each sampling was measured by using a digital electronic balance (Mega Digital Scale; precision=0.1 gm). At the end of the experiment, all the ponds were dewatered by water pump and all the prawn was harvested completely to determine the weight gain, SGR, survival rate, FCR and other production performance parameters.

i) Weight gain (g):

Weight gain = Mean final weight – Mean initial weight

ii) Percent weight gain (%):

$$\% \text{ Weight gain} = \frac{\text{Mean final prawn weight} - \text{Mean initial prawn weight}}{\text{Mean initial weight}} \times 100$$

iii) Specific growth rate (% per day):

$$\text{SGR (\% per day)} = \frac{\ln W_2 - \ln W_1}{T2 - T1} \times 100$$

Where,

W_1= Initial live body weight (g) at time T_1 (day)

W_2= Final live body weight (g) at time T_2(day)

iv) Survival rate (%):

$$\text{Survival Rate (\%)} = \frac{\text{No. of total live prawn}}{\text{Total no. of prawn stocked}} \times 100$$

v) FCR (Feed Conversion Ratio):

$$\text{FCR} = \frac{\text{Total feed consumed(kg)}}{\text{Total yield(kg)}}$$

vi) Production:

The net production of prawn (Kg/decimal) was calculated by the following formula:

$$\text{Net Production (kg/decimal)} = \frac{\text{Survival rate} \times \text{Stocking density} \times \text{Weight gain (gm)}}{100 \times 1000}$$

Statistical analysis

Recorded data were analyzed using Microsoft excel 2010. Data analysis was done by using the SPSS software (Statistical Package for Social Science) version-20 according to Duncan's New Multiple Range Test (Duncan, 1995) to identify the 5% level of significance of variance among the different treatment means.

Results

Water quality parameters

In the present study, important water quality parameters, like temperature, pH, alkalinity, dissolved oxygen (DO), transparency and hardness were monitored and presented in Table-1.

Table 1. Mean (±SD) values of water quality parameters measured from different treatments.

Parameters	Treatments			Level of Significance
	T1	T2	T3	
Temperature (°C)	25.41±4.63[a]	25.35±4.58[a]	25.59±4.58[a]	NS
pH	7.76±0.3[a]	7.79±0.35[a]	7.84±0.31[a]	NS
Alkalinity (mg/l)	115.87±9.3[a]	115.86±9.51[a]	117±9.86[a]	NS
DO (mg/l)	4.14±0.26[a]	4.09±0.29[a]	4.14±0.25[a]	NS
Transparency (cm)	33.37±3.2[a]	33.02±3.02[a]	32.74±2.91[a]	NS
Hardness (ppm)	190.73±8.31[a]	191.99±7.47[a]	193.42±5.3[a]	NS

NS= Means are not significantly different (p>0.05).

All the water quality parameters found within the acceptable range for freshwater prawn culture (FAO, 2002). The pH of the control pond and treatment ponds were 7.76±0.3, 7.79±0.35 and 7.84±0.31 respectively.

The temperature (25.41±4.63- 25.59±4.58°C) of both control and treatment ponds were closely similar. The dissolved oxygen, alkalinity, transparency and hardness was more or less similar. The statistical analysis showed that there was no significant difference (P>0.05) among three treatments.

Growth performance parameters

Growth, Survival rate and production of *M. rosenbergii* after 150days of culture can be seen in Table2 and the average weight of prawn in each sampling is shown in Fig. 1.

There were no significant differences of final mean body weight, mean weight gain and SGR among all the treatments (P>0.05) (Table 2). After culture period, the highest mean weight gain (52.8±2.88 g) was found in T3 in which the feed probiotics were used. The lowest final weight of prawn (40.13±3.9 g)

was found in T1 (control) and the highest final weight was found in treatment T_3 (54.73±2.87g).The mean specific growth rates in weight were 2.02±1.51, 2.16±1.65 and 2.23±1.87% body weight in T_1, T_2 and T_3 treatments, respectively. The highest value was obtained from T_3 and the lowest value was obtained from $T_1$and other probiotics treated ponds also showed better SGR than the control ponds. There was no significant difference (P>0.05) in specific growth rates of prawn among different treatments.At the end of experiment the survival rate of prawn was highest in T_3 (93.86±1.42%) and lowest in T_1(control) (79.55±1.78%) and there was significant difference among the treatments (P<0.05).

The mean Feed conversion ratios were 2.27±0.43, 1.31±0.08 and 1.13±0.15 in T_1, T_2 and T_3 treatments, respectively. The highest value was obtained from T_1 (2.27) and the lowest value was obtained from T_3 (1.13). There was significant difference (P<0.05) in FCR of prawn among the treatments (Table 2).

Table 2. Growth performances of prawn in different treatments (Mean ±SD).

Characters	Treatments			Level of Significance
	T1	T2	T3	
Mean initial weight (gm)	1.93±0.06 [a]	1.93±0.06 [a]	1.93±0.06 [a]	NS
Mean final weight (gm)	40.13±3.9 [a]	49.13±4.23 [a]	54.73±2.87 [a]	NS
Mean weight gain (gm)	38.2±3.92 [a]	47.2±4.23 [a]	52.8±2.88[a]	NS
SGR (% body weight)	2.02±1.51 [a]	2.16±1.65 [a]	2.23±1.87 [a]	NS
Survival rate (%)	79.55±1.78 [a]	89.39±1.51 [b]	93.86±1.42 [c]	*
FCR	2.27±0.43 [a]	1.31±0.08 [a]	1.13±0.15 [b]	*
Net production (kg/bigha)	667.76±58.25 [a]	928.08±80.79 [a]	1090.27±60.65 [a]	*

NS= Means are not significantly different (p>0.05)

*= Means are significantly different (p≤0.05).

The mean net productions of fresh water prawn were 667.76±58.25, 928.08±80.79 and 1090.27±60.65kg/hain T_1, T_2 and T_3 treatments, respectively. The highest value was obtained from T_3 (1090.27±60.65kg/ha) and the lowest value was obtained from T_1 (667.76±58.25kg/ha). There were significant differences (P<0.01) in net production of prawn among the three treatments.

Discussion

The result of the present study showed that the effect of different probiotics plays an important role on growth, survival and production of freshwater prawn *M. rosenbergii* by improving and maintaining good water quality of the culture ponds. Temperature, dissolved oxygen, pH are important water quality parameters considered during the study period. Water quality parameters were found more suitable in the ponds where probiotics used than in controlled ponds those matches with the report of Jiravanichpaisal *et al.*(1997).Previous studies showed that different water and soil quality parameters have an influence on the sustainable production of shrimp (Ramanathan *et al.* 2005; Soundarapandian and Gunalan 2008). In the present study pH was 7.76±0.3 in controlled ponds 7.79±0.35-7.84±0.31 in probiotic treated ponds. Ramanathan *et al.* (2005) said that the optimum range of pH 6.8 to 8.7 should be maintained for maximum growth and production. As per Gilles (2001)we know dissolved oxygen plays an important role on growth and production through its direct effect on feed consumption and maturation and low levels of dissolved oxygen can cause damages in the oxidation state of substances from the oxidized to the reduced form. Dissolved oxygen level in water of experimental ponds as recorded in the present study agreed well with findings of Wahab *et al.,* (1995). In the present study dissolved oxygen was found 4.14 ppm and 4.09-4.14 ppm in controlled and probiotic ponds respectively. Soundarapandian *et al.* (2010)

found 3.2 to 4.2 ppm dissolved oxygen in all their culture ponds that is close to the present study. Low-level of oxygen hampers metabolic performances in shrimp and can reduce growth and moulting and also causes mortality (Gilles, 2001). Temperature is one of the most important a biotic factor affecting both growth and survival of crustaceans. In this study temperature was found 25.41°C and 25.35-25.59°C in controlled and probiotic ponds respectively which was maintained within reference ranges. So, it was found although the experiment that probiotic doesn't alter water temperature or it does not have any beneficial effect on it. Ling, (1969) recommended the range of temperature from 22°C to 32°C is optimum for growth of fresh water prawn that was similar to the case of present study. Fujimura, (1974) and Sandifer *et al.,* (1983) recorded optimum temperatures for prawn have been reported to range from 27°C to 33°C.To meet up the increasing demand for fish and for environment-friendly aquaculture, the use of probiotics in aquaculture is now considered alternative to antibiotics and chemicals to improve the quality and sustainability of aquaculture production. Since the first use of probiotics in aquaculture, a growing number of studies have demonstrated their ability to control potential pathogens and to increase the growth rates and welfare of farmed aquatic animals (Gatesoupe, 1991; Carnevali *et al.*, 2004; Macey and Coyne,2005; Wang *et al.*, 2005; Wang and Xu, 2006). During the culture period in the experiment, all the probiotics treated pond resulted in an increase of final weight and weight gain of prawn.

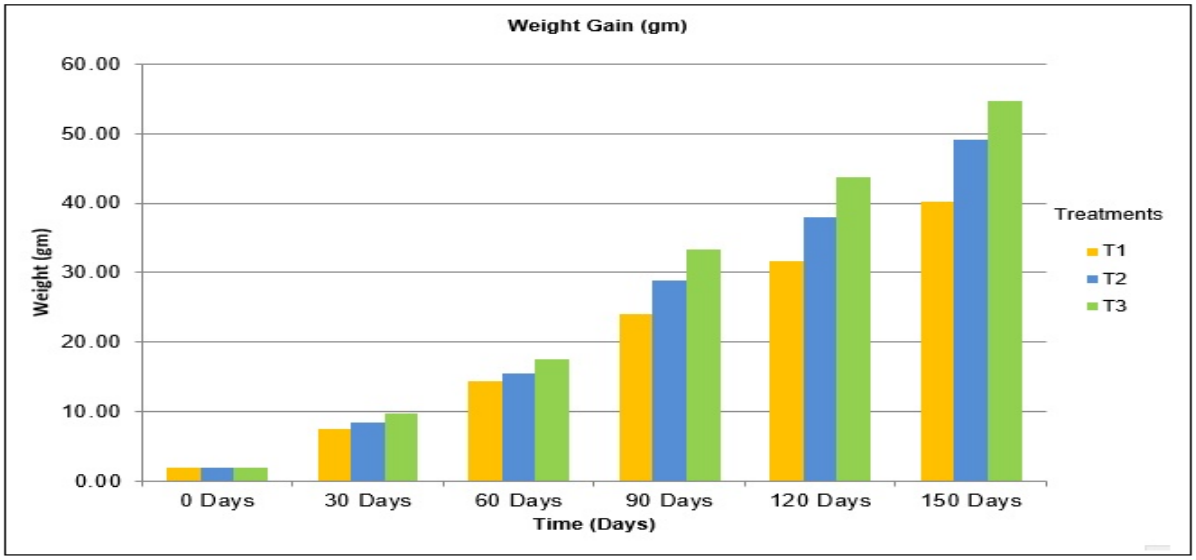

Fig. 1. Comparison of weight gain of prawns in different treatments during experimental period.

This is a very inspiring result for prawn culture with probiotics because the high-quality prawn is related with the market price and for the foreign earnings. This study is almost similar the study of Saad *et al.* (2009) and Hossain *et al.* (2013) in which better growth was found for probiotics treated shrimp and prawn than without probiotics treated shrimp. Gullian *et al.* (2004) stated a significant growth in shrimp that was treated with Bacillus sp. In some studies, the survival rate of post larvae (PL) of prawn was significantly higher in probiotics treated prawns than control (Seenivasan *et al.*, 2011; Hossain *et al.*, 2013). The present study was similar to those studies. Rinisha *et al.* (2010) observed probiotics effects onthe growth and survival of *Macrobrachium rosenbergii* post larvae and after 90 days they observed 76% survivability where probiotics was introduced through feed, whereas 64% survivability observed in the control. At the end of experiment the survival rate of prawn was highest in probiotic ponds (93.86±1.42%) and lowest in controlled ponds (79.55±1.78%) and there was significant difference among the treatments ($P < 0.05$) and this result supports the above studies. The highest FCR (2.27) was found in T_1which differed

significantly. The present study supported findings of Irianto and Austin (2002) where they revealed probiotics bacteria as a good candidate for improving the digestion of nutrients and growth of aquatic organisms. The probiotic bacteria have been shown to improve the digestive activity by producing digestive enzyme and enhanced digestion and increased absorption of food which ultimately improved feed utilization efficiency and low FCR(Lee and Lee, 1990; Gatesoupe, 1999; El-Dakar and Goher, 2004; Ziaei-Nejad *et al.*, 2006). The net production was higherin probiotics treated ponds(T_3) than control.

The per hectare total production was 1090.27±60.65kg in T_3 than T_1which supports the study of Hossain *et al.* (2013). The highest production in T_3 might cause of faster growth rate, higher final weight, higher survival rate, better culture friendly water quality parameters due to functioning of highest number of beneficial bacteria. In these circumstances, to increase our prawn production in comparing withother developing countries, application of commercial probiotics is essential.

Conclusion

From this experiment it can be generally concluded that probiotics had significant effect on growth, survival and production of prawns. The production was always higher for probiotics treated ponds than other without probiotics treated ponds.

The findings of this study could be applied to the farmer's level to increase the production of prawn using probiotics thus to increase the profits and to minimize the losses.

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