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Application of probiotics and prebiotics for promoting growth of Tiger shrimp (*Penaeus monodon*): an approach to eco-friendly shrimp aquaculture

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ABSTRACT

The current study has been conducted to evaluate the growth performance of shrimp (*Penaeus monodon*) by applying eco-friendly culture mechanism like prebiotics and probiotics. The experiment was carried out for 95 days in different shrimp farms at coastal district of Bagerhat, Bangladesh. Three different treatments viz., probiotic treated as T1, prebiotics treated as T2 and both probiotics and prebiotics as T3 with a control group were designed to conduct the experiment. The size of the experimental ponds was five acre and the stocking density was 4/m^2 in each treatment. CP NASA shrimp feed (32% protein) was given thrice in a day during the study period. After 95 days of culture period, the maximum weight gain was observed at T3 (33.78±0.18 g) whereas the minimum weight gain was observed at control group (25.69±0.10 g). The survival rate was the highest in T3 (89.01%) followed by T2 (75.51%) and T1 (53.44%) and the lowest rate was observed in control group (50.88%). Overall production was higher in T3 (833.78 kg ha^{-1}) compared to T2 (553.40 kg ha^{-1}), T1 (447.84 kg ha^{-1}) and Control group (310.57 kg ha^{-1}). pH value was found to maximum in T3 (7.71±0.08) and it was minimum in T1 (7.41±0.10). In addition, the maximum TAN value was found to be 2.22±0.19 mg L^{-1} in C pond and it was minimum in T3 (0.32±0.06 mg L^{-1}). Therefore, it could be concluded that combine application of probiotics and prebiotics might be the reliable media to enhance production of shrimp by maintaining eco-friendly environment in aquaculture.

Keywords: Probiotics, Prebiotics, Eco-friendly, Growth, Shrimp.

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Introduction

The shrimp sector in Bangladesh is very unique compared to other fisheries and supported large coastal fishing industrial development (Howlader *et al.*, 2020). More than 95% of shrimp and prawn in Bangladesh are produced in extensive polyculture ponds that were formerly used as rice ponds and are locally referred to as 'Ghers'. The culture of *Penaeus monodon* (black tiger shrimp), locally referred to as 'Bagda', is mostly combined with the culture of other shrimp species that are trapped in the gher when water is taken in (DoF, 2017; Ghosh, 2018; Howlader *et al.*, 2020). The culture of *Macrobrachium rosenbergii* (Giant freshwater prawn) takes place in smaller ponds and is mostly combined with the culture of rice and/or freshwater fishes (Ghosh *et al.*, 2016). *M. rosenbergii* is mostly (over 95%) produced by small-scale farmers, who harvest only a few kg of large shrimp per day. Shrimp exports total over 40,000 tonnes of *P. monodon, M. rosenbergii* and some smaller volumes of other species in 2016 and 2017 (Azad *et al.*, 2019). Over 80% was exported to the EU market in 2017, especially to the Netherlands, Germany and Belgium. There are more than 70 factories approved by the Department of Fisheries (DoF) in Bangladesh, but only 40-50 are operational (FAO, 2013; Hossain *et al.*, 2013).

Shrimp and prawn together represent the second largest exportable items contributing to foreign exchange earnings of Bangladesh (Ghosh *et al.*, 2016; Azad *et al.*, 2019). Shrimp farming has significant impact on environment and economy. Although the country has a great history of shrimp/prawn farming, the productivity of shrimp is very low compare to the other shrimp producing countries of the world (Rahman and Rashid, 2013). One of the major causes of poor productivity is the extensive or traditional method of farming (90 percent of total farms), whereas developed countries brought their farms under intensive or semi-intensive methods of farming (Azad *et al.*, 2019). Bangladesh has great potentiality to increase productivity of shrimp and prawn by introducing intensive and semi-intensive methods of farming (Rahman and Rashid, 2013).

Although shrimp industries having a great contribution in the economy of Bangladesh as well many developing countries (Alam and Ahammad, 2017). But, the industries have facing lot of challenge in recent decays. As a result, production and quality of shrimp commodities are also declining gradually (Ghosh, 2018). Now a day the current aquaculture moved to eco-friendly culture system (Shingare *et al.*, 2020). Different types of environment friendly culture methods have been developed. Probiotics and prebiotics are one of the most popular eco-friendly culture systems around the world (Kumar *et al.*, 2016; Shefat, 2018; Shingare *et al.*, 2020). Probiotic are heterotrophic beneficial bacteria that remove deleterious nitrogenous compound from the aquatic system (Fernandes and Kerkar, 2019). The principal mode of action of probiotic bacteria is through competitive exclusion mechanisms in which pathogens are replaced or excluded through the development of a beneficial microbial population on the intestinal surface which leads to a reduction in disease, better health and thus better growth of the host (Jha, 2014; Azad *et al.*, 2019).

Thus, probiotics act as the alternatives of antibiotics and chemicals reducing diseases through maintaining pollution free environment (Kumar *et al.*, 2016; Fernandes and Kerkar, 2019). On the other hand, prebiotics acts as free carbon source that helps heterotrophic bacteria to digest ammonia. It also ensures enough oxygen, stabilize the pH and balance the density of algae-bacteria ratio.

Considering that aspect, current study was conducted to focus the effect of probiotics and prebiotics on shrimp production in the southern coastal region of Bangladesh. The current research focused on assessing growth performance of shrimp through eco-friendly culture practices with the application of probiotics and prebiotics.

Methodology

Experimental site and size

Field experiment was carried out directly on the farmers' commercial shrimp farms. A total 12 commercial farms were selected. The average area of the pond was with 1.5 m depth and the farmers mostly practices conventional shrimp farming without the application of probiotics and prebiotics.

Experimental species

The experiment only focused on Shrimp monoculture techniques. Specific Pathogen Free (SPF) shrimp larvae *Penaeus monodon* was cultured and observed their growth performance by applying different probiotics and prebiotics. Shrimp post larvae (PL) of 15 days old was collected from a commercial hatchery located at Cox's Bazar district of Bangladesh.

Description of probiotics and its application

Three different commercial probiotics were selected considering on their function, compositions, mood of application such as soil, water and gut probiotics and obviously on their economics aspect (Table 1).

Table 1. Information about applied probiotics with its composition and mode of application.

Type of probiotics	Microbial composition	Manufacturing company	Marketing agency	Mode of application
Soil probiotics	*Rhodobacter* sp. and *Rhodococcus*	Kyushu Medical Co. Ltd, Japan	Fish-Tech	7 days prior of PL stocking at dosage 150 ml/decimal and continue with 10 days interval during the culture period at dosage 100 ml/decimal.
Water probiotics	*Bacillus subtilis* and *Bacillus licheniformis*	INVE (Thailand) Ltd	INVE Aquaculture	7 days prior of PL stocking at dosage 0.5 g/decimal and continue with 10 days interval during the culture period at dosage 1.0 g/decimal.
Gut probiotics	*B. mensentericus*, *Bacillus subtilis* and *Licheniformis*, *Nitrobacter* sp.	Murdoch University, Australia & Inje University	UniBioCare (BD) Corporation	Mix with daily feed for shrimp at dosage 1-2 g/kg feed.

Preparation of prebiotics

Prebiotics was prepared by using locally available ingredients such as rice bran/auto polish, molasses, yeast powder these are the major component that is very available near the hand (Table 2). In this process rice bran, molasses and yeast powder is taken in required amount and mixed in water. The amount of water should be 10 times more than the total mixture weight. Then the mixture is kept in a plastic drum or other jar that must have lid to cover and it needs at least 24-72 hours to ferment. After the fermentation the solution must be filtered with small meshed cloths or net and collect only the water that should be sprinkled all over the farm water. Each time of application, newly prepared prebiotics should be applied.

Table 2. Ingredients used to prepare prebiotics and their specification with functions.

Ingredients & support materials	Amount/Acre	Specification
Rice polish (Auto Polish)	4 kg	Source of carbohydrates.
Molasses	4 kg	Fructooligosaccharides. Help to increase microbial and plankton number.
Yeast Powder	100 g	Single cell micro-organism of the *Saccharomyces cerevisae* used for fermentation and important source of carbon-di-oxide.
Water	10 times than the mixture	Help to mix all ingredients properly with pond environment.

Experiment set up

The experiment was conducted with four treatments including a control. The treatment group were expressed as T_1 (with only prebiotics, T_2 (using only probiotics), T_3 (using both prebiotics and probiotics) and C as control pond using none of these. All other management like stocking density ($4/m^2$) feed, feeding methods, liming etc. were similar throughout the experimental period.

Feeding management

Commercial supplementary feed formulated by CP India Ltd for shrimp was used containing fish meal, shrimp head meal, cod liver-oil, squid meal, broken rice, soybean meal, wheat flour, cholesterol, phospholipids, vitamins and minerals (Leaflet of CP Feed). Before feeding, the feed was analysed to determine the proximate composition according to the standard procedures given by Association of Official Analytical Chemists (AOAC, 1980). The average percentage of protein, fat and moisture was 31, 7 and 12%, respectively in the feed. Feed was given according to the body weight and the age of shrimp. At first month, feed was given at 10% of total shrimp body weight, and then up to five months feed was given at 5% of body weight, and last two months the feed was given at 2% of the total body weight of shrimp (the average weight of shrimp was multiplied by the total number of shrimp to calculate total weight) (Table 3). Feed was spread over the pond surface three times in a day at 30% (6.00 am), 30% (12.00 pm) and 40% (6.00 pm) of the total feed allocated for the shrimp.

Table 0. Feed Chart followed in treatment (CPF-2017, India) for 1000 individual.

Feed used/1000 biomass						
Age (Days)	Average body weight (g)	Weight of 1000 shrimp (kg)	Feeding ratio (% body weight)	Daily feed (g)	Total feed (kg)/week	Feeding frequency/day
1-7	0.8	0.8	10.0	0.080	0.560	3
8-14	1.5	1.5	9.0	0.135	0.945	3
15-21	4.0	4.0	5.5	0.220	1.540	3
22-28	6.0	6.0	5.0	0.300	2.100	3
29-35	8.5	8.5	4.4	0.374	2.618	3
36-42	10.0	10.0	4.1	0.410	2.870	3
43-49	12.0	12.0	3.8	0.456	3.192	3
50-56	15.0	15.0	3.4	0.510	3.570	3
57-63	18.0	18.0	3.0	0.540	3.780	3
64-70	21.5	21.5	2.6	0.559	3.913	3
71-77	24.0	24.0	2.4	0.576	4.032	3
78-84	27.0	27.0	2.3	0.621	4.347	3
85-91	30.0	30.0	2.2	0.660	4.620	3

Sampling and growth measurement

The culture was done for 95 days. Sampling was done regularly at every week. The water salinity of the pond was measured by using a hand Refractometer (Erma-Japan). The pH, alkalinity, total ammonia nitrogen (TAN) of the pond water was recorded by using pH test kit, alkalinity kit and ammonia test kit, respectively (Advance Pharma, Thailand). Water temperature was measured by using a standard centigrade thermometer.

Statistical analysis

All data were analysed statistically using SPSS version 16.0, Chicago, SPSS lnc. Normality test done by Shapiro–Wilk to check for normal distribution and homogeneity of variance. Only percent data had to be arcsine transformed before analysis; however, non-transformed data are presented in tables. Least significance difference (LSD) post hoc test on a one-way ANOVA was used to examine treatment effects on weight gain. All statistical analyses were considered at 5% ($p<0.05$) level of significance.

Results and Discussion

Growth, survival rate and production of P. monodon

There were significant differences observed of the final mean body weight of shrimp among all treatment ($p<0.05$) (Table 4). After 95 days of culture period the highest mean body weight was found at T3 (33.78±0.18 g) in which both probiotics and prebiotics were applied combined whereas lowest mean growth was in T4 (25.69±0.10 g) the control group (Table 0). Similarly, Significant effect of prebiotics and probiotics also observed on survival rate of shrimp among all treatment ($p<0.05$). The maximum survival rate was found at T3 (89.01%) whereas at control group the rate was almost half of the initial stocking. However, the overall production at T3 almost three time more than the control.

Table 0. Body weight, % survival rate and production (mean ± standard error) of *P. monodon* treated with three different prebiotics and probiotics.

	C	T1	T2	T3
Initial Weight (g)	0.74±0.01	0.77±0.01	0.81±.01	1.08±0.01
Final Weight (g)	26.43±0.10	27.81±0.10	30.91±0.13	34.85±0.18
Weight gain (g)	25.69±0.10[a]	27.04±0.11[a]	30.10±0.13[a]	33.78±0.18[a]
Survival rate (%)	50.88±2.66	53.44±0.95	75.51±2.06	89.01±1.90
Production (kg/ha)	310.56±81.20	447.84±21.08	553.40±50.81	833.78±87.96

Different superscript letters (effect of prebiotics and probiotics) indicate significant difference among the treatments (One-way analysis of variance, p<0.05).

It is clearly observed from this experiment that the probiotics and prebiotics enhanced overall shrimp production. Although the combine application of probiotics and prebiotics dramatically help to get higher production. Similar scenario production performance by probiotic application also observed by Shingare *et al.*, (2020) in Maharashtra India in shrimp polyculture, Ghosh *et al.*, (2016) at Khulna region of Bangladesh in Prawn monoculture and Shefat, (2018) at shrimp farm in southern part Bangladesh.

Observation of probiotics and prebiotics interaction effect on individual growth variables

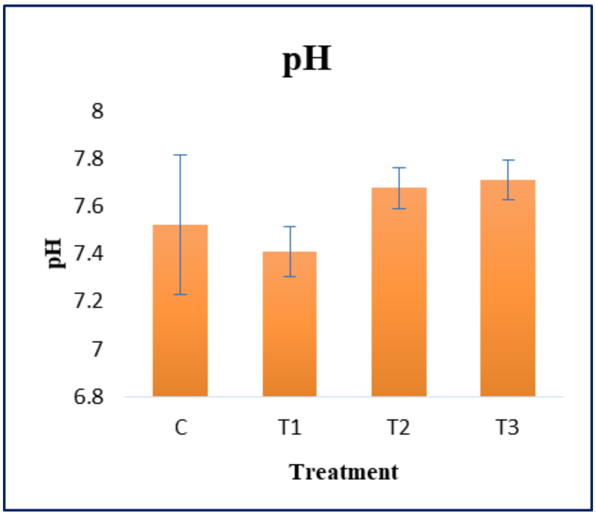
The LSD post hoc test on a one-way ANOVA revealed that there was a statistically significant difference in body weight gain between the group of the control and all the treatments ($p = 0.00$) (Table 5).

Table 5. Analysis of Variance (ANOVA).

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
IW	Between Groups	2.107	3	0.702	83.728	0.000
	Within Groups	0.973	116	0.008		
	Total	3.080	119			
FW	Between Groups	1257.900	3	419.300	748.789	0.000
	Within Groups	64.957	116	0.560		
	Total	1322.857	119			
WG	Between Groups	1163.272	3	387.757	677.350	0.000
	Within Groups	66.406	116	0.572		
	Total	1229.678	119			

Water quality parameter in experimental pond

pH value was found to be maximum in T3 (7.71±0.08) and minimum in T1 (7.41±0.10) (Figure 1). In addition, the maximum TAN value was found to be 2.22±0.19 mg L^{-1} in C pond and minimum in T3 (0.32±0.06 mg L^{-1}) (Figure 1). From different earlier studies the normal pH of brackish water ranged between 7 to 9 for culturing shrimps and less than 7 or higher than 9 often found harmful to shrimp, even is detrimental to the health of the shrimps (Muthu, 1980). In the present study, the pH concentration was ranged from 7.41±0.10 to 7.71±0.08, which were favorable for fish and shellfish culture (Ghosh *et al.*, 2016). At farm level, Ammonia level should be less than 1 ppm (Soundarapandian *et al.*, 2010). In the present study, total ammonia nitrogen was 2.22±0.19 and 0.32±0.06 in control and prebiotics probiotics treated ponds, respectively. Thus, maintaining the ammonia level probiotic and prebiotics help in maintaining good water quality and thereby keeps the shrimp disease free (Hossain *et al.*, 2013; Azad *et al.*, 2019).



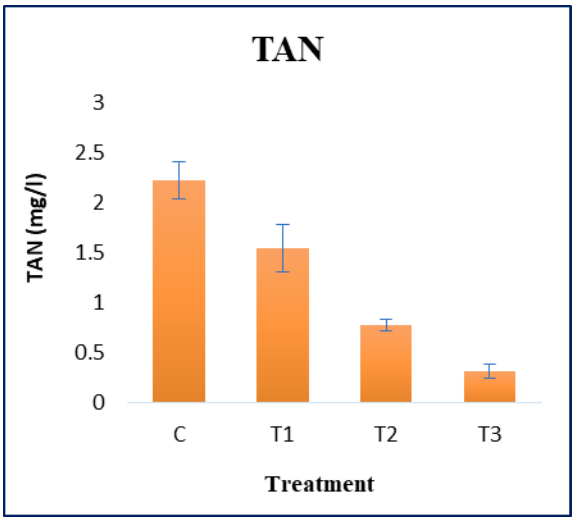


Figure 1. pH and TAN values in all ponds during the 3 months experimental period. The error bars indicate the standard error of the treatments.

Most often economic analysis is an important issue for this kind of study. Along with the production we also conducted cost benefits analysis and we found that our profit if almost double then the cost of experiments. Therefore, we can say applying both prebiotics and probiotics during the culture period, these components act as stress remover as well as decrease the pathogenic activity, that might increase the survival rate and enhance shrimp production along with the economically profitable eco-friendly aquaculture.

Conclusion

This study confirmed that highest shrimp growth and production was found in that treatment where both probiotics and prebiotics were used combined. As well probiotics increase survival rate of the species and produce healthy crops within three months. Along with this these ingredients plays a significant contribution to maintain suitable water quality especially main good TAN and pH, which ensure ecofriendly pond environment and minimizing different kind of diseases. Therefore, it could be concluded that application of both prebiotics and probiotic in shrimp farm can be a profitable and ecofriendly culture techniques for coastal shrimp farmer.

References

Alam, S. and Ahammad, S.U. 2017. Shrimp aquaculture in Bangladesh: Domestic regulatory responses and compliance with MEAs. *Marine Policy*. 82: 122–133. https://doi.org/10.1016/j.marpol.2017.04.018

AOAC. 1980. *Official methods of analysis* (13th ed.). Association of Official Analytical Chemists. Washington D.C. pp. 376-384.

Azad, M.A.K., Islam, S.S., Sithi, I.N., Ghosh, A.K., Banu, G.R., Bir, J. and Huq, K.A. 2019. Effect of probiotics on immune competence of giant freshwater prawn *Macrobrachium rosenbergii*. *Aqua. Res.* 50(2): 644–657. https://doi.org/10.1111/are.13942

DoF. 2017. Yearbook of Fisheries Statistics of Bangladesh 2016-17. Fisheries Resources Survey System (FRSS), Department of Fisheries. Bangladesh : Director General, DoF, vol. 34, p. 129.

FAO. 2013. Characteristics, structure and resources of the sector. *FAO Country Notes, 1380*, Rome, Italy. pp. 1–16.

Fernandes, S. and Kerkar, S. 2019. Bacterial probiotics over antibiotics: A boon to aquaculture. (eds. S.N. Meena and M.M. Naik) *In:* Advances in Biological Science Research. Academic Press, Elsevier. pp. 215-232. https://doi.org/10.1016/B978-0-12-817497-5.00014-8

Ghosh, A.K. 2018. Effect of feeding level on growth, body composition, fatty acid profile, and nutrient accumulation in shrimp (*Litopenaeus vannamei*). *Aqua. Int.* 26: 405–417. https://doi.org/10.1007/s10499-017-0225-z

Ghosh, A.K., Bir, J., Azad, M.A.K., Hasanuzzaman, A.F.M., Islam, M.S. and Huq, K.A. 2016. Impact of commercial probiotics application on growth and production of giant fresh water prawn (*Macrobrachium Rosenbergii* De Man, 1879). *Aqua. Rep.* 4: 112–117. https://doi.org/10.1016/j.aqrep.2016.08.001

Hossain, M.I., Kamal, M.M., Mannan, M.A., Bhuyain, M.A.B. and Hossain, M.I. 2013. Effects of probiotics on growth and survival of shrimp (*Penaeus monodon*) in coastal pond at Khulna, Bangladesh. *J. Sci. Res.* 5(2): 363-370. https://doi.org/10.3329/jsr.v5i2.11815

Howlader, P., Ghosh, A.K., Islam, S.S., Bir, J. and Banu, G.R. 2020. Antiviral activity of *Cynodon dactylon* on white spot syndrome virus (WSSV)-infected shrimp: an attempt to mitigate risk in shrimp farming. *Aqua. Int.* 28: 1725-1738. https://doi.org/10.1007/s10499-020-00553-w

Jha, A.K. 2014. Probiotics: an effective feed supplement in Shrimp culture. *J. Bangladesh Acad. Sci.* 38(2): 127–130. https://doi.org/10.3329/jbas.v38i2.21336

Kumar, V., Roy, S., Meena, D.K. and Sarkar, U.K. 2016. Application of probiotics in shrimp aquaculture: importance, mechanisms of action, and methods of administration. *Rev. Fish. Sci. Aqua.* 24(4): 342–368. https://doi.org/10.1080/23308249.2016.1193841

Muthu, M.S. 1980. Site selection and type of farms for coastal aquaculture of prawns. pp. 16–18. *In:* Proceedings of the Symposium on Shrimp Farming, Bombay, India.

Rahman, M.C. and Rashid, M.H.A. 2013. A case study on the present status and potentiality of shrimp farming in Bangladesh. *Asia-Pacific J. Rural Dev.* 23(1): 97–110. https://doi.org/10.1177/1018529120130107

Shefat, S.H.T. 2018. Use of probiotics in shrimp aquaculture in Bangladesh, *Acta Sci. Microbiol.* 1(11): 20–27.

Shingare, P.E., Pagarkar, A.U., Chaudhari, K.J., Dhaker, H.S., Meshram, S.J., Sawant, N.H., Satam, S.B., Shingare, S.P., Sawant, B.T. and Sapkale, P.H. 2020. Eco-friendly and sustainable Asian seabass culture system: an alternate candidate species other than shrimp for brackish water aquaculture. *J. Exp. Zool.* 23 (Suppl. 1): 983–985.

Soundarapandian, P., Ramanan, V. and Dinakaran, G.K. 2010. Effect of probiotics on the growth and survival of *Penaeus monodon* (Fabricius). *Current Res. J. Soc. Sci.* 2(2): 51–57.