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PROBIOTIC EFFECTS ON TIGER SHRIMP (*PENAEUS MONODON*) GROWTH AND WATER-SEDIMENT QUALITY OF GHERS IN BAGHERHAT, BANGLADESH

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Abstract

Probiotics are live microorganisms which enhance shrimp productivity and maintaining favourable water and soil qualities. This study was conducted to ascertain the effect of three probiotics on shrimp growth, feed utilization, survival, water and sediment qualities in coastal ponds of Bagerhat, Bangladesh. Twelve ponds were selected and divided into four treatment groups as T_1 (Miracure), T_2 (Biozyme), T_3 (Rapidgrow) and T_4 (control-without probiotics) with same stocking densities of 6 PL/m². Shrimp were feed 30% protein (Mega feed Ltd.) thrice daily at 5-10% of body weight for 120 days. Growth performance, shrimp biomass and feed utilization increased significantly ($p<0.05$) in probiotics treated groups, especially Miracure, compared with the control. It was observed that probiotic treated ponds showed significantly improved shrimp growth performance (32.2-38.5%), survival (14-18%) and lower FCR (33.5-38.7%) compared to control ponds. NH_3 decreased significantly ($p<0.05$) in probiotics treated ponds compared to control ponds. Significant improvement of sediment parameters (organic matter, total nitrogen, phosphorus and potassium) was observed in probiotics treated ponds. Probiotic application also exhibits water and sediment qualities by higher total heterotrophic bacteria (THB) and lower *Vibrio* species, contributing to overall health status of the shrimp ponds. This study reinforces the importance of probiotics as a long-lasting adoption for enhancing shrimp farming in Bangladesh. All future research should be focused on the effects of probiotics. The extent of benefits is the curtail of chemicals used in aquaculture at the coastal and marine environment.

Key words: White gold, Feed conversion ratio, Total heterotrophic bacteria, Organic matter, Total nitrogen, Phosphorus

Introduction

Coastal aquaculture, encompassing both shrimp/prawn and finfish farming, has been expanding in Bangladesh's coastal regions. Efforts have also been directed at establishing a business-friendly supply chain with a strong focus on hygiene and safety standards for fish, shrimp and fishery products destined for both domestic and international markets.

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These interventions have led to a rise in total shellfish production and prawn production, including both wild and farming, 175,000 metric tons in 2003-04 to 271,000 metric tons in 2022-23 (DoF, 2024). Shrimp farming holds significant prominence due to its economic value and widespread cultivation across coastal regions of the world. In this regard, the black tiger shrimp (*Penaeus monodon*) emerges as the key commercial species, making a significant contribution to the global seafood export (DoF, 2024). The GDP growth in fisheries sector of Bangladesh in 2024 was 2.45 percent and the contribution of the fisheries sector in the overall agriculture sector was 21.68 percent (DoF, 2024).

For shrimp farming in particular, beneficial bacteria namely probiotics are emerging as an effective alternative to antibiotics. Probiotics are usually referred to as live microbial feed additives that help in maintaining a healthy microflora in the environment (Fuller, 1989). They can be used in aquaculture either as food additives or in water treatment (Moriarty, 1998). Probiotics use in aquaculture serve various functions, including inhibiting pathogenic bacteria by producing antimicrobial compounds, improving water parameters, boosting defense mechanism of the vector organism, and supplementing the vector's diet through production of additional enzymes (Verschuere *et al*., 2000). Studies have demonstrated that the lactic acid bacteria can increase the survival of shrimp exposed to *Vibrio harveyi* (Vieira *et al*., 2010).

Probiotic use in shrimp culture has been a subject of great interest over the last decade. A number of studies have been operated to explore the significance of probiotics on growth, survival, disease resistance, and water quality in various farming systems (Pal *et al*., 2010). In aquaculture, research on probiotics has primarily focused on in vitro models to assess their effectiveness in combating pathogens (Wang *et al*., 2008). These supplements enhance nutrient utilization by providing enzymes that improve the digestibility of dietary components, thereby facilitating better nutrient absorption in the host. As the push for environmentally sustainable aquaculture continues to grow, probiotics are increasingly recognized as a vital component of such systems (Moriarty, 1998; Gatessoupe, 1999). Although several commercial probiotics such ProbioAqua, Magic, SP super and Miracure, are available both liquid and granulated forms, many farmers, especially in Bangladesh, lack sufficient knowledge about their proper use and benefits. Islam *et al.* (2013) has explored the application of probiotics in shrimp farming within brackish water environments as broader utilization of probiotics in Bangladesh's fish farms.

The range of commercial probiotic products used in aquaculture around the world, whether as feed or water additives, showed a great deal of variation in terms of their effectiveness, pricing, and significant rates of ineffectiveness in some of the items. The effective use of probiotics in aquaculture, however, depends in large part on the method of administration (Jahangiri and Esteban, 2018) and the probionts. Non-beneficial probiotics may even be detrimental to fish/shrimp species; in addition, they may result in greater financial losses (Hai, 2015). This highlights the need for investigation into the effectiveness of probiotics in diets, particularly for shrimp farming. Considering the issue, this research had been undertaken to assess the effects of probiotics on shrimp growth performance, output and water-sediment qualities in farming system in coastal Bangladesh.

Materials and Methods

Area of study and period: The research work was performed in twelve brackish water shrimp ghers in the Bagerhat Sadar, Bagerhat. The ponds were of an average size of 400 m^2, ranging from 0.9 to 1.6 meters in depth. The duration of the research was one culture period, March - July 2023.

Experimental design: The experiment included four treatments: T_1 (Miracure), T_2 (Biozyme), T_3 (Rapid grow), and T_4 (without probiotics) each applied in three replicates. Stocking density of shrimp PL was six (6) individual per square meter. Dose of selected probiotics was 0.5 g per kg of supplied feed in each treatment without T_4. Probiotics mixed with feed very properly and carefully, and then supplied to the experimental ponds.

Preparation of waterbodies and management of water: Each of ponds was left to dry as well as crack such that the hydrogen sulfide can be oxidized and kill fish eggs, crab larvae, snail larvae, and other predators. The top 30 cm of surface soil was removed from each pond to release harmful gases, oxygenate the bottom soil, and improve soil fertility. Using a cone-type pH meter (Hanna 8424), the soil pH was measured, averaging 6.8 across the ponds. To neutralize the acidic soil and increase nutrient availability, lime (CaO) was used at a dose of 250 kg/ha. Waterbodies were subsequently fulled tidal water from Bhairab river to a depth of 60 cm, allowing one or two days for sedimentation. Afterward, ponds were treated with fish killing substance (rotenone-3 ppm) to eliminate trashed organism, followed by lime (CaO) at 125 kg/ha to neutralize the rotenone's effect. Following 5 days of cleaning, ponds were inoculated with urea and TSP (urea- 50 kg/ha and TSP-100 kg/ha) after being cleaned for five days. Water turned green after four to five days. Around ponds, fine mesh nylon net was placed to guard against potential carriers of disease that could enter into shrimp ponds, like snails, snakes, and other animals. 25-50 kg/ha of lime (CaO) was added with water of culture ponds from time to time every 7 to 15 days to adjust the pH level during the culturing period.

Shrimp PL stocking: All ponds were stocked with hatchery produced post larvae (PL) of *Penaeus monodon* (average body weight, 0.006 g) at a uniform density of 6 individuals per square meter. Prior to stocking, the PL were carefully acclimated to the pond water. Shrimp PL were purchased from Foyla bazar in Rampal, Bagerhat. Shrimp PL were transferred from market to the study location in oxygenated polythene bags. Bags submerged in the research waterbodies for a period of thirty minutes to permit water circulation among the pond and the bags to adjust the PL to pond water temperature prior to releasing the PL into the ponds. The PL were stocked in the experimental ponds after acclimatization. The weight and length of 30% of the PL were moved separately using digital balance and scale and released to the ponds.

Supplement diet: The stocked shrimp post-larvae fed a Mega feed from gradually lowering amount, starting at ten percent of their total weight and reducing to 5%, thrice a day at 06:00 am, 13:00 pm and 19:00 pm until the day before harvest. The composition of the supplied feed was moisture-11%, crude protein-30%, crude lipid -7%, ash- 15%, crude fiber-8% and carbohydrate- 29%.

Feeding protocol and growth measurement: Commercial Mega floating feed was used as the food for the shrimps. The feed started at 10% of the body mass of the shrimps and slowly reduced to 5% over the duration of the study. Quantity of feed was splited into three portions given equally on a daily basis at specific intervals: morning, noon and evening. Feed levels were alternated each 15 days according to body weight fluctuation of the shrimp. Shrimp behavior was carefully monitored, especially following morning and evening feeding, to assess their condition, including movement, infection, coloration, and diseases, if any. Net used to assess shrimp growth, with fortnightly sampling to record the individual number and their body weight. Five to seven hauls were conducted in each pond to monitor shrimp health, signs of disease, color, and average body weight. Sampling continued regularly until harvest.

Probiotics selection, water and sediment qualities monitoring: Three brand names of probiotics as Miracure, Rapid Grow, and Biozyme were selected for the experiment. These were sourced from Fish Tech Pharmaceuticals, located at Shahjalal Upashahar, Sylhet. Environmental parameters such temperature, transparency, pH, salinity, DO, total alkalinity as well as ammonia nitrogen were analyzed every 7 days at 10 am of each sampling excursion. Salinity was determined using a portable refractometer (ATAGO, Hand Refractometer) and pH was determined through pH meter (Hanna Instruments, HI 8010, Japan). In situ temperature of water was measured with a common Celsius thermometer, and the DO concentration was recorded by DO meter (YSI 58 digital DO meter, HANNA, Yellow Springs, Ohio 45387 USA). Transparency was found through a Secchi disc. Total alkalinity was analyzed by titrimetric methods (APHA, 1992), and ammonia concentration calorimetrically determined, as described by Strickland and Parsons (1968) and APHA (1992). Sediment cores were collected from all the ponds at three sampling sites during culture: initial (before stocking), mid-term (60-day culture), and final term (120-day culture). The samples were dried under the air, ground into granulated form, and stored in airtight bags. These analysed for pH, organic matter, nitrogen, phosphorus, and potassium in the laboratory of the Soil Research Development Institute (SRDI), Sylhet.

Bacteria estimation: The total heterotrophic bacteria (THB) and *Vibrio* species loads were assessed monthly. Water and soil samples aseptically collected through various ponds promptly transported to research place. The THB load was determined through the pour plate method using plate count agar as the culture medium, following the standard protocol outlined in APHA (1992) in the Water and Soil Quality Lab of Shrimp Research Station, BFRI, Bagerhat. The load of *Vibrio* species was also measured using the pour plate technique, employing thiosulfate citrate bile salts (TCBS) agar medium. The bacterial load in each sample was calculated with a standard formula and reported as colony-forming units (CFU) per milliliter or gram of the sample.

Harvesting and growth parameters: After a 120-day culture period, leaves and bamboo poles were taken out and ponds drained for harvest of shrimp through frequent netting operations (seine net plus cast net). Every shrimp taken from each pond was individually counted, measured, and weighed. Survival rate (%), specific growth rate (SGR) and food conversion ratio (FCR) were calculated following the equation as cited by Pechsiri and Yakupitiyage (2005).

The equations are as follows:

Status of growth and feed utilization as weight gain = (Mean final weight (g) − Mean initial weight (g),

SGR = {Ln (final body weight)−Ln (initial body weight) × 100}/cultured period (days)

FCR = (Feed consumed (g dry weight)/live weight gain (g wet weight) of shrimp)

Survival rate (%) = (No. of shrimps harvested ÷ Total number of shrimps stocked) × 100.)

Production of shrimp = (No. of shrimp caught × Average final weight of shrimp) were calculated.

Statistical analysis: The data collected throughout the experimental period were recorded, stored and then analyzed using one-way analysis of variance (ANOVA). Tukey's post hoc test ($p<0.05$) was used (IBM SPSS Statistic 20 software) to identify significant differences among all the treatments. Results were expressed as the mean standard error and the difference was considered significant at $p<0.05$.

Results and Discussion

Growth performance and production, and feed utilization: The use of probiotics in shrimp ponds was considerably ($p<0.05$) beneficial. In comparison to the control treatment, all probiotic-treated treatments showed enhanced growth performance and production (Table 1). The best significant ($p<0.05$) results were obtained by the Miracure product (T_1). In the majority of cases, T_1, T_2, and T_3 produced findings that were similar without significant differences ($p<0.05$). The probiotics application significantly ($p<0.05$) improved feed utilization indices of shrimp in all probiotics-treated ponds compared with the control ponds. Treatment 1 achieved the best FCR values compared with the other treatments. But T_1, T_2 and T_3 gave similar values for FCR without significant differences among them ($p > 0.05$). Treatment 1 reduced FCR values by 38.7% compared with the control, while the reduction percent was 35.6 and 33.5% for T_2 and T_3, respectively. Treatment 1 achieved 39.6% higher values for SGR compared to the control treatment (T_4). Also, T_2 and T_3 obtained 37.5% and 39.8% higher values for SGR, respectively, compared to T_4. Compared to the control treatment, T_1 had a 18% significantly greater survival rate. However, there was no discernible difference in survival between T_2 (15%), and T_3 (14%). Shrimp production was statistically higher in T_1, T_2, and T_3 compared to control (T_4). There was a notable variation in production among the four treatments but variation was not found between T_2 and T_3.

The welfare and performance of farmed organisms are directly impacted by the quality of the aquatic environment (Hura *et al.*, 2018). Probiotics considerably enhanced the growth performance and feed utilization of the tiger shrimp in the current study as compared to the control treatment. The Miracure treatment produced the best outcomes. The positive effects of probiotics treatments are in agreement with many studies in shrimp, *P. monodon* and marine fishes. Shefat (2018) stated that, in aquaculture, probiotics have a substantial effect on the immune system, growth rate, and feed utilization capacity of shrimp, prawns, crabs, and finfish. Pal *et al.* (2010) found that growth and production of

P. monodon in probiotic-treated ponds were superior to those in the control ones. Probiotics are beneficial for shrimp (*P. monodon*) growth and output, but they are essential to determine the right and profitable stocking density, according to Ghosh *et al.* (2013). For marine fishes, Aly *et al.* (2016) noticed that EM® probiotic significantly boosted the final weight and specific growth rate of European seabass fry compared with the control treatment with 26.7% improvement. Additionally, Lotfy (2015) revealed that probiotics to the rearing water of *Sparus aurata* increased ADG by 36.7% when compared to the control. El-Okaby (2015) concluded that *Sparus aurata*, the fingerlings' growth performance, feed consumption, condition variables, and survival percentage were all better in probiotics treated ponds than those of the control. The beneficial role of beneficial microorganisms in improving the digestive system (Thiam *et al.*, 2015; Lotfy, 2015), stimulating appetite, producing vitamins, breaking down indigestible nutrients (Irianto and Austin, 2002), lowering pathogenic microbes in the gastrointestinal tract (Lotfy, 2015), and ultimately improving feed absorption (Tovar *et al.*, 2002) may be the reason for the positive effect of probiotics in improving the nutritional status of fish and shell fish species.

Table 1. Effects of probiotics on growth performance and feed utilization of studied tiger shrimp for 120 days culture.

Species and production parameters	Treatments			
	T_1 (Miracure)	T_2 (Biozyme)	T_3 (Rapid grow)	T_4 (Control)
Average initial weight (g)	0.006 ± 0.001	0.006 ± 0.001	0.006 ± 0.00	0.006±0.001
Average final weight (g)	19.12 ±1.02^{a}	18.90±0.87^{a}	18.25± 1.05^{a}	13.80±1.11^{b}
Daily weight gain (g)	0.16± 0.02^{a}	0.15 ± 0.02^{a}	0.15± 0.01^{a}	0.11±0.01^{b}
Food conversion ratio (%/day)	2.10±0.55^{b}	2.21±0.53^{b}	2.28±0.60^{b}	3.43±0.59^{a}
Specific growth rate (%/day)	7.19±0.18^{a}	7.08±0.19^{a}	7.20±0.17^{a}	5.15±0.10^{b}
Survival rate (%)	78.00 ± 03^{a}	76.00 ± 05^{a}	75.00 ± 04^{a}	66.00±06^{b}
Production (kg/ha)	894.82± 94.69^{a}	861.84 ± 74.81^{a}	821.30 ± 59.31^{ab}	546.48±29.38^{c}

Values are presented as mean ± SD (n=3); means in row bearing different superscript letters differ significantly ($p<0.05$).

The beneficial effects of probiotics of the present work is coincided with many researchers for shell fish, catfish and other species like Pacific white shrimp *Penaeus vannamei* (Xia and Zhu, 2014), Gilthead sea bream *Sparus aurata* (Lotfy, 2015), European sea bass *Dicentrachus labrax* (Aly *et al.*, 2016)), yellow perch *Perca flavescens* (Shaheen *et al.*, 2014*)*, Asian catfish *Pangasius hypophthalmus* (Gobi *et al*., 2016), Abalone *Haliotis discus hannai* (Guo *et al*., 2017), tilapia *O. niloticus* (El-Kady *et* *al*., 2022, Madani *et al*., 2018; Sutthi *et al*., 2018; Wang *et al*., 2017; Wang *et al*., 2008b; Zhou *et al*., 2010) and stinging catfish *Heteropneustes fossilis* (Sohel *et al*., 2023) . The FCR and growth performance of *O. niloticus* were significantly improved by *Bacillus subtilis*, *B. licheniformis*, and combination. *Bacillus* species have a dose-dependent capacity to improve growth performance (Elsabagh *et al*., 2018; Madani *et al*., 2018). Shahriar *et al.* (2024) demonstrated that adding commercial probiotics that are readily available in the area to *A. testudineus* diets enhanced resistance, ematobiochemical indices, nonspecific immunity, and growth and feed utilization performance.

Water quality: Application of probiotics as a water additive showed significant (p<0.05) improvement in water quality parameters (Table 2). The values of water transparency and alkalinity of probiotics treated ponds were significantly different from control ponds. The lowest value of ammonia nitrogen (NH_3) was in favor of T_1 (Miracure product). The control treatment exhibited the highest levels of NH_3. The probiotics-treated treatments showed a decreasing ratio in NH_3 values, with rates varying between 51.5–58.6%, compared with the control treatment.

Table 2. Effects of probiotics on water quality parameters of tiger shrimp experimental ponds for 120 days culture.

Parameters	Treatments			
	T_1 (Miracure probiotics)	T_2 (Biozyme probiotics)	T_3 (Rapid grow probiotics)	T_4 (Control)
Temperature (ºC)	29.05 ± 1.83	29.10 ± 1.79	29.12 ± 2.003	31.06±1.98
Water transparency (cm)	28.15 ± 2.55 [a]	28.30 ± 2.71 [a]	28.41± 2.37 [a]	41.66±2.46 [b]
Dissolved oxygen (mg / l)	4.84 ± 0.28	4.79 ± 0.27	4.77 ± 0.28	4.02±0.27
Salinity (ppt)	4.74 ± 0.79	4.66 ± 0.82	4.73 ± 0.77	4.71±0.76
pH	7.54 ± 0.20	7.50 ± 0.20	7.48 ± 0.21	6.65±0.19
Total alkalinity (mg / l)	105.92 ± 3.47 [a]	102.87 ± 3.66 [a]	99.83 ± 3.47 [a]	80.12±3.46 [b]
Ammonia nitrogen (mg / l)	0.041 ± 0.026 [a]	0.045 ± 0.025 [a]	0.048 ± 0.024 [a]	0.099±0.025[b]

Values with a different alphabetical superscripts in the same row differ significantly (p<0.05). All values are expressed as mean ± SD (n=3).

According to a number of studies, probiotics improve feed digestion, boost the immune system, and increase growth and survival in aquaculture (Huerta-R´abago *et al*., 2019). The majority of scholars looking into how probiotics affect aquaculture have so far employed nutritional supplements. The possible advantages of administering probiotics in water, however, have received little consideration (Jahangiri and Esteban, 2018). Probiotics have been developed in laboratories, but there are now commercial products on the market. *Bacillus* isolates are among the essential bacterial species present in the majority of commercial products (Martínez Cruz *et al*., 2012). A number of variables, including water temperature, transparency, DO, salinity, pH, alkalinity, ammonia, probiotic source, dosage, inoculation period, and fish/shrimp age, might influence the effectiveness of probiotics administered through water (Jahangiri and Esteban, 2018, El-Kady *et al*., 2022). Water qualities of the treatment ponds of tiger shrimp stayed well in comparison to control ponds (Pal *et al*., 2010).

The current study's results show a significant improvement in water quality, as seen by a notable drop in the levels of harmful ammonia in ponds treated with probiotics. The treated ponds' transparency and alkalinity differed greatly from the control ponds. Such findings are consistent with other earlier research (Balcazar *et al.*, 2006; Banerjee and Ray, 2017; Jahangiri and Esteban, 2018; Jozwiakowski *et al*., 2009; Zheng *et al*., 2011; Zhang *et al*., 2011). When the denitrifying bacteria *Bacillus licheniformis* was added to fish rearing water, toxic chemicals (TAN and NH_3) reduced and the rate at which protein and starch in leftover or uneaten feed degraded rose, according to Zheng *et al*. (2011). Wastes were mineralized as a result of *Bacillus* genera' biodegradation process (nitrification and/or denitrification) of nitrogenous by-products, which also improved the quality of the water (Zhang *et al*., 2011).

It has been shown that Bacillus strains are useful for clearing organic materials from aquatic training ponds (Luis-Villase˜nor *et al*., 2011). Zorriehzahra *et al*. (2016) stated that gram-negative bacteria can turn organic materials into bacterial biomass, while gram-positive bacteria-specifically *Bacillus subtilis*-can effectively convert organic matter to carbon dioxide, gram negative bacteria can convert organic matter into bacteria biomass. Probiotics like *Bacillus* sp. added to rearing water would boost beneficial bacteria and proliferate them in fish/shrimp ponds (Kuebutornye *et al*., 2019). Zhu *et al*. (2019) therefore came to the conclusion that within seven days of the bacterial addition, *Melampodium gracile* YL28 absorbed 95.6% of ammonium and eliminated about 99.3-99.96% of nitrite from the water. Ammonia also dropped in ponds treated with probiotics for Nile tilapia (Wang *et al*., 2017) and grass carp *Ctenopharyngodon idellus* (Zhang *et al*., 2011). Regarding the advantages of introducing probiotics to marine environments, Aly *et al*. (2016) obtained that, when compared to the control treatment, the EM® probiotic reduced the amount of TAN and NH_3 in the rearing tanks of European seabass fry, with an ammonia removal efficiency of 66.28%. El-Kady *et al*. (2022) found that probiotic-treated groups, particularly EM®, showed a significant ($p<0.05$) drop in TAN and NH_3 when compared to the control. The control group had higher TAN and NH_3 concentrations. Comparing the probiotic-treated groups to the control group, the ratio of

TAN and NH_3 values decreased, with rates ranging from 43 to 64% and 52.6-32.3%, respectively. Xu *et al*. (2013) reported that *Bacillus* sp. can create extracellular enzymes and antimicrobial peptides that inhibit pathogenic bacteria and enhance water quality, which is another important advantage of adding probiotics to water.

Sediments quality: After the probiotics application, nutrient level in sediments such organic matter, total nitrogen, phosphorus and potassium was significantly decreased (Table 3). The amount of organic matter (OM) in probiotics treated ponds was significantly lower of 31.5-34.7% compared to control ponds. The concentrations of total nitrogen (TN), phosphorus (P) and potassium (K) in treated ponds were significantly decreased ($p<0.05$) than that of control ponds. The decreasing rate of TN, P and K contents in treated ponds were of 25.6-29.6, 58.0-59.1 and 27.8-33.3%, respectively compared to control ponds.

Table 3. Effects of probiotics on sediment qualities of experimental shrimp ponds.

Parameters	Treatments			
	T_1 (Miracure)	T_2 (Biozyme)	T_3 (Rapid grow)	T_4 (Control)
pH	7.88±0.08	7.84±0.07	7.80±0.06	6.18±0.05
Salinity (Ec) (dS/m)	13.26±4.69	13.32±4.84	13.50±5.13	13.52±5.15
Organic matter (%)	1.43±0.16 ^{a}	1.45±0.12 ^{a}	1.50±0.16 ^{a}	2.19 ±0.17 ^{b}
Total nitrogen (%)	0.088±0.008 ^{a}	0.091±0.007 ^{a}	0.093±0.006 ^{a}	0.125±0.005 ^{b}
Phosphorus (µg/g)	8.04±3.36 ^{a}	8.09±2.67 ^{a}	8.12±3.38 ^{a}	11.43±3.35 ^{b}
Potassium (meq/100 g)	0.60±0.04 ^{a}	0.63±0.02 ^{a}	0.65±0.01 ^{a}	0.90±0.01 ^{b}

Values with a different superscripts in the same row are significantly different ($p<0.05$). All values are expressed as mean ± SD (n=3).

By holding onto or releasing nutrients, pond sediment contributes significantly to the cycling of nutrients. The use of probiotic in aquaculture ponds was strongly recommended by Moriarty (1996). In a shrimp farm in West Java, Suhendra *et al*. (1997) found that regular usage of commercial probiotics led to improved environmental conditions, less organic matter accumulation, and better water quality. Following the ponds' treatment with commercial probiotics, the study's data revealed lower quantities of OM, TN, P, and K in the sediment. So, it was determined that the probiotics enhanced the ecology of the shrimp pond and had a significant impact on nutrient cycling.

The lowering of sediment nutrient levels in the shrimp ponds under study was consistent with a prior study that found that probiotic additions significantly ($p<0.05$) reduced the levels of total organic carbon (TOC), total nitrogen, phosphorus, and total inorganic phosphorus (TIP) in the sediment (Matias *et al*., 2002; Wang *et al*., 2005; Wang and He, 2009). Boyd *et al*. (1994) and NACA (1995) stated that soil conditions are more crucial than water conditions in influencing brackish water productivity. Similarly, Chakrabarti *et al*. (1985) suggested that, just as temperature and salinity in the water phase affect shrimp production, organic carbon, available nitrogen, and phosphorus in the soil phase also have a direct relationship with shrimp production.

Bacterial load: The quantitative load of total heterotrophic bacteria (THB) increased and simultaneously the quantity of *Vibrio* sp. decreased in all probiotics treated compared to without probiotic ponds (control). The bacterial population varied with each sampling, consistently showing higher levels in sediment compared to water in both ponds treated with probiotics and control ponds (Figs 1-4). By the end of the culture period, bacterial populations increased in the probiotic-treated ponds. The proportion of *Vibrio* spp. relative to the THB in the control ponds did not exhibit a significant decline. *Vibrio* spp. contributed substantially to the THB load in the control ponds, whereas the probiotic-treated ponds demonstrated a lower abundance of *Vibrio* spp. The outcomes of this study are close to Dalmin *et al*., 2001; Pal *et al*. (2010) and Islam *et al*. (2013). The current

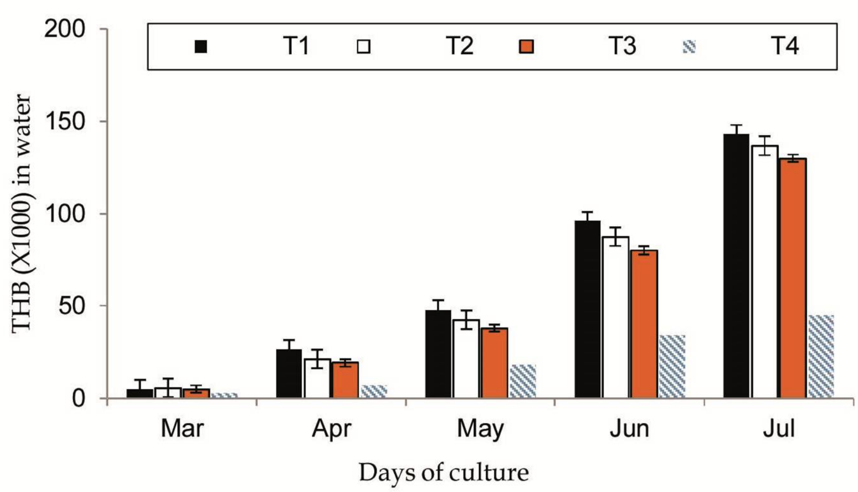

Fig. 1. Quantity of THB in shrimp ponds water with and without probiotics in different treatments.

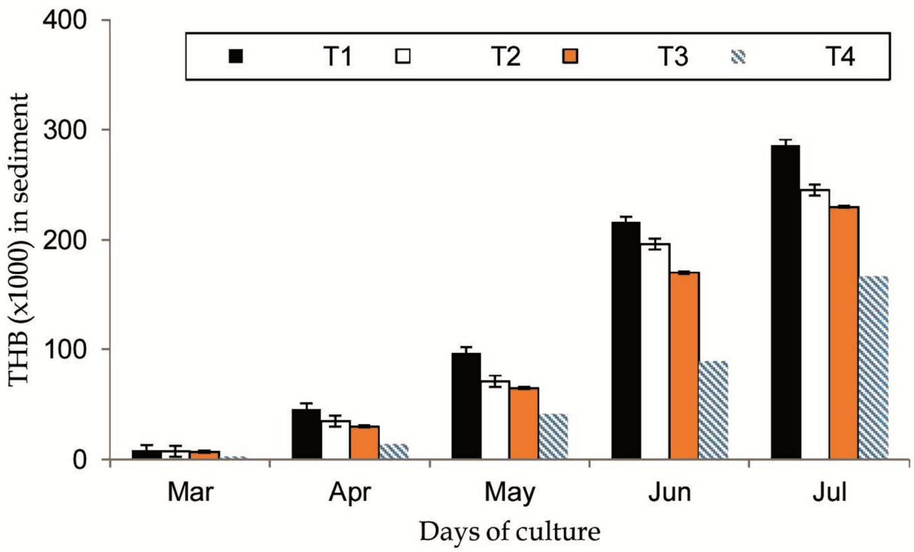

Fig. 2. Quantity of THB in shrimp ponds sediment with and without probiotics in different treatments.

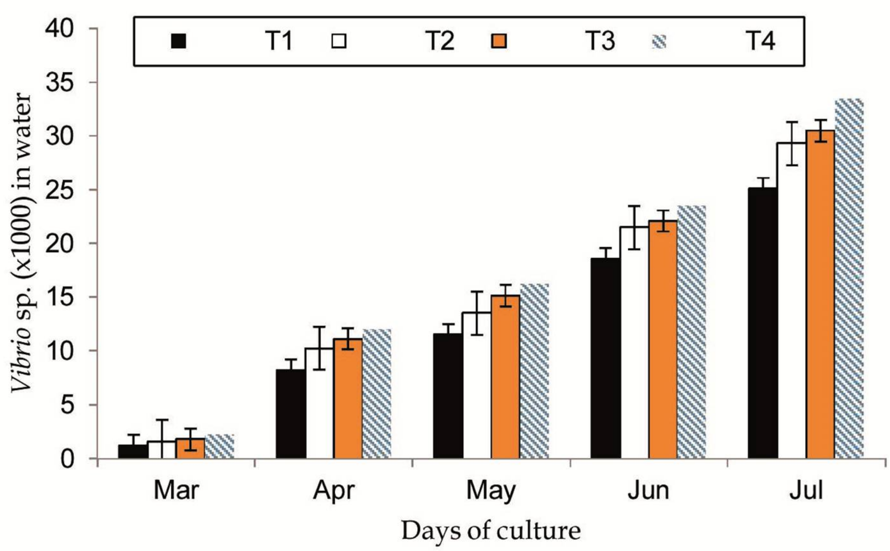

Fig. 3. Quantity of *Vibrio* sp. in shrimp ponds water with and without probiotics in different treatments.

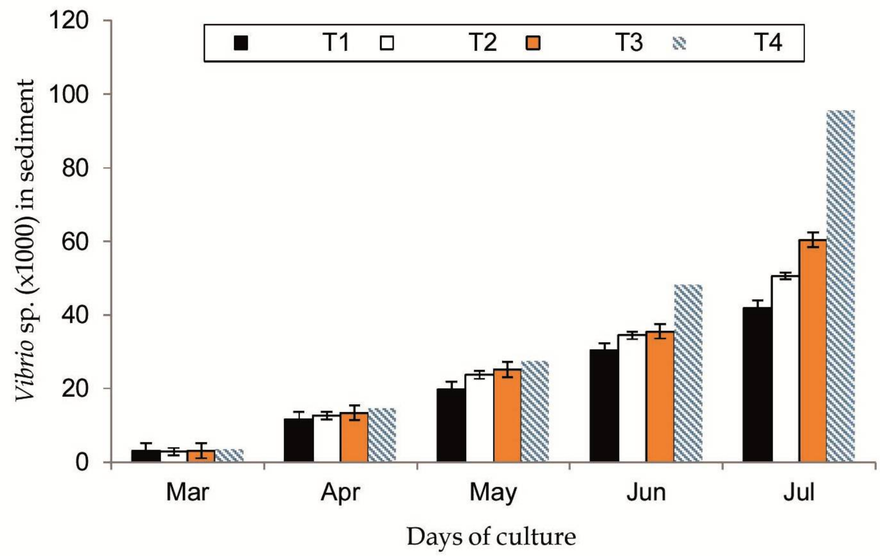

Fig. 4. Quantity of *Vibrio* sp. in shrimp ponds sediment with and without probiotics in different treatments.

probiotic study is associated with better shrimp growth, water quality, and fewer pathogenic microorganisms. Because of the actions of autochthonous flora such as *Flavobacterium*, *Micrococcus*, and *Alcaligenes* that are present in the ecosystem, the probiotic's 10^{10} CFU/ml *Bacillus* spp. number dropped to 10^{3} CFU/ml after being applied in the pond. The vibrios' burden also significantly decreased at the same time (Westerdahl *et al.*, 1991).

Conclusion

The effectiveness of commercial probiotics as an additive to shrimp growth and to enhance the quality of water and sediment varies greatly. The application of small amounts of commercial probiotics in shrimp ponds produced numerous positive results, including an increase in growth performance of 32.2-38.5%, a decrease in FCR of 33.5-38.7%, an improvement in survival rate of 14-18%, and a reduction in the ammonia content in rearing water of 51.5-58.6%. The application of probiotics significantly reduced the amount of nutrients in pond sediment such as OM by 31.5-34.7%, TN 25.6-29.6%, P 58.0-59.1%, and K 27.8-33.3%, which were utilized to boost shrimp growth and improve the water quality. The findings indicate the superiority of Miracure over Rapid grow and Biozyme. The use of commercial probiotics increased the amount of

THB and decreased the amount of *Vibrio* sp. Overall, the study supports the importance of probiotics as a water additive to enhance shrimp growth performance, water and sediment quality, though further research is still required to optimize commercial products and apply them properly, which will improve the aquatic environment for shrimp farming and production in ponds.

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