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Aquamimicry improves the growth performance and immunity of black tiger shrimp (*Penaeus monodon*) in low saline ponds

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ABSTRACT

Shrimp farming is currently a great challenge because of unexpected diseases and increasing prices of commercial feed. This study was conducted to evaluate the effects of the aquamimicry culture technique, based on the application of fermented rice bran as a replacement for commercial feed, on the growth, immunity, and survival rate of black tiger shrimp (*Penaeus monodon*) reared under natural conditions to address these challenges. Aquamimicry culture ponds were designed with high-density polythene liners in the embankment to prevent soil erosion, and a central pit with a suction pump to remove accumulated culture waste, while traditional existing ponds were prepared following standard methods. The liquid fermented rice bran (LFRB) was used to produce food before stocking. Shrimp were grown for 90 days in one of three treatments: T_0 (control):100% commercial feed (CF) in a traditional pond, T_1:90% CF + 10% LFRB, or T_2:70% CF + 30% LFRB in aquamimicry pond with a density of 10 PL/m^2. LFRB was prepared by fermenting rice bran with *Bacillus subtilis* for 24 h under continuous aeration. The average growth rate of shrimp was significantly higher in T_2 (0.47 g day^{-1}) than in T_1 (0.34 g day^{-1}) or T_0 (0.05 g day^{-1}). The survival rate of the shrimp was significantly higher in treatments T_2 (55 ±12%) and T_1 (45±8%) than in treatment T_0. Furthermore, *Vibrio parahaemolyticus*, the pathogenic strain responsible for early mortality syndrome in *P. monodon*, was identified based on bacterial colony morphology obtained from the water and hepatopancreas of shrimp in the control ponds. The shrimp in the T_2 treatment had a healthier hepatopancreas and significantly higher total hemocyte count (3.6×10^3cells mL^{-1}) than the shrimp reared in T_0 (2.5×10^3cells mL^{-1}) and T_1(2.5×10^3cells mL^{-1}). This study revealed that the aquamimicry culture technique could ensure better growth, immunity, and survival rates of black tiger shrimp, whereas 70% CF + 30% LFRB (i.e., T_2) showed the best performance.

1. Introduction

Shrimp farming has become an important economic activity and the largest export industry in many aquaculture-dominant countries worldwide, owing to its significant role in fulfilling protein demand and food security. Shrimp accounts for approximately 16.4% of the global trade in fish and fishery products in terms of value, making them the second most significant commercial food commodity after tuna (FAO, 2022). *Penaeus monodon*, a member of the Penaeid shrimp family, has significant economic value as a traded species and is popularly referred to as the black tiger shrimp throughout the Indo-Pacific region (Rao et al., 2013). The production of *P. monodon* reached 717.1 thousand tonnes, representing 6.4% of total crustaceans' output in 2020 (FAO, 2022).

However, shrimp culture practices require a transformation from extensive to semi- or somewhat intensive techniques to obtain the highest production from limited resources. Shrimp are reared at high stocking densities, and a large amount of feed is used to obtain more

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production from a unit area in intensive aquaculture (Ali et al., 2022). Shrimp farming is threatened by unexpected diseases caused by abnormal pond environments, climatic conditions, and excessive use of commercial feed and chemicals due to intensification (Hasan et al., 2020; Jamal et al., 2023). Notably, the present scenario is compounded by the emergence of unforeseen diseases, such as early mortality syndrome (EMS), predominantly caused by *Vibrio parahaemolyticus*, resulting in sudden and significant black tiger shrimp mortality outbreaks (Hossain et al., 2023). Nowadays, traditional aquaculture farmers also face problems owing to the high feed cost, making it difficult for them to sustain their practices and compete with modern methods. Low-cost and eco-based shrimp culture technologies must be developed to increase the production's economic and environmental sustainability (Castro et al., 2021). Moreover, Oligohaline environments present distinct osmoregulatory demands on shrimp populations, necessitating careful management strategies to mitigate stress and optimize growth (Molina et al., 2019). Eco-friendly technologies are now practiced to decrease commercial feed use and ensure the shrimp industry's sustainable development (Khanjani et al., 2024).

Aquamimicry is one of the advanced and sustainable technologies in the shrimp farming industry that stimulates natural conditions by applying natural prebiotics, e.g., fermented carbohydrates, to generate live food, especially "Copepod," for the post larvae before stocking (Khanjani et al., 2022; Santos et al., 2022). Fermented carbohydrate sources, such as fermented rice bran, are the most effective feed supplements because of their effective roles as pre- and probiotics (Yang et al., 2021; Haryati et al., 2015). The fermentation process subsequently improves the water solubility, palatability, nutrient digestibility, and immune function of the feed (Abdel-Tawwab et al., 2022). The aquamimicry technique, which incorporates fermented rice bran, serves two essential functions. First, it creates bioflocs to maintain good water quality by creating heterotrophic microbial community formation and stabilization(Emerenciano et al., 2017). These microorganisms help to improve the water quality of a system by removing inorganic nitrogen from wastewater by uptaking of nitrogen compounds generating in situ microbial protein(Emerenciano et al., 2017; Nisar et al., 2022). Second, it supports pond zooplankton and provides supplemental nutrition to shrimp (Khanjani et al., 2023). In addition to the post-larvae directly feeding on live feeds, it is believed that the larger and benthic shrimp consume zooplankton's eggs when they sink to the pond bottom (Romano et al., 2018). Natural foods contain several vital components, including proteins, lipids, carbohydrates, amino acids, vitamins, minerals, and fatty acids. Consequently, it is often referred to as "living capsules of nutrition" (New, 1998). Moreover, the carbon source provides a balanced carbon: nitrogen (C:N) ratio to heterotrophic microbes for growth and function; thus, toxic nitrogenous metabolites such as ammonia are converted into microbial proteins (Avnimelech, 2009). Therefore, fermented carbohydrates and probiotics used in aquamimicry systems could significantly minimize the cost of feed in shrimp farming (Ekasari et al., 2014; Karaki et al., 2016; Supriyati et al., 2015).

In addition, aquamimicry technology provides a disease-free culture environment and emphasizes the natural remediation of ecosystems by promoting the natural cycling of minerals and the production of natural food organisms (Romano and Kumar, 2017). This technique significantly reduces water exchange and high-stocking-intensive cultures (Nisar et al., 2022). It provides low-cost, sustainable aquaculture production and high-quality outputs by reusing organic nutrients (Romano et al., 2018; Cho and Yiğit, 2022).

A few studies (Catalani et al., 2023; Liñan-Vidriales et al., 2021) have been conducted on aquamimicry systems, focusing on Pacific white shrimp (*Litopenaeus vannamei*) culture in where they compared the Biofloc with Aquamimicry technology and evaluated the effect of fermented rice bran on dynamic microbial activity.To our knowledge, this is the first study to examine the effects of an aqumimicry culture technique using probiotic-enriched fermented rice bran as a natural feed stimulator on the growth, immunity, and survival of black tiger shrimp (*Penaeus monodon*) raised in a natural culture environment. The specific objectives include assessing zootechnical performance, analyzing the microbial status, and evaluating the overall sustainability and viability of the aquamimicry system in shrimp farming. Through these objectives, this study seeks to enhance understanding of the aquamimicry system's importance and contribute essential knowledge for advancing sustainable culturing practices in shrimp farming.

2. Materials and methods

2.1. Design of aquamimicry pond

A field experiment was conducted at a shrimp farm situated in the coastal region of Bangladesh. The analysis was performed at the Department of Fisheries Biology and Aquatic Environment Laboratory, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Bangladesh. Existing traditional ponds and Aquamimicry ponds with a size of 1214 m^2 were excavated. The volume of each pond was approximately 1850 $m^{3.}$ Aquamimicry ponds were designed using high-density polyethylene (HDPE) liners in the embankment and a suction pump at the central pit (Fig. 1). HDPE liners were set up to prevent soil erosion, and the central pit was excavated to remove the accumulated culture waste. Each treatment consisted of three replicates, and nine ponds were used in the experiment. The pond embankment was fenced to maintain biosecurity. Two 1HP aerators were placed at the two corners of the aqua-mimicry pond.

The key differences between aquamimicry and existing traditional ponds are listed in Table 1.

2.2. Fermentation of rice bran

Before fermentation, husk-free rice bran was ground and sieved into smaller pieces. Five liters of water was mixed with one kg of ground rice bran and incubated for 24 h under continuous aeration for fermentation. A separate shed was prepared, and a blower was used for aeration. A commercial probiotic named Red Cap (Baxel Company Limited, Thailand), containing *Bacillus subtilis, Bacillus amyloliquefaciens*, *and Bacillus valismortis* at a concentration of 2×10^{10} colony-forming units (CFU) per gram, was used for fermentation. Before fermentation pH was measured using Digital pH meter (Hach) and it was adjusted at 6.0–7.0 with calcium carbonate. This was achieved by gradually introducing calcium carbonate while continuously monitoring the pH.

2.3. Pond preparation

The steps taken during pond preparation for natural food production in the aquaculture ponds are listed in Table 2; none of these procedures were followed for the control ponds. Two probiotics, Super PS (Feedwale, India) and Superbiotic (CPF Private Limited, India), containing *Rhodobacter* spp. and *Rodococcus* spp., with a concentration of $3{\times}10^8$ cfu were used in this process. As stated in the package labeling, these bacteria operate in a synergistic relationship to biodegrade organic pollutants, reduce hydrogen sulphide and toxic gases. Natural feed development in ponds was confirmed by observing the color of the water and plankton density in the laboratory.

2.4. Experimental design of shrimp culture

The experiment was split into three treatments, each with three replicates, based on the pond preparation and feed application mode (Table 3). Specific pathogen-free (SPF) post larvae (PL) of black tiger shrimp (*P. monodon*) were obtained from a hatchery and verified using PCR analysis at the Shrimp Research Station, Bagerhat, Bangladesh. Then, shrimp PL was acclimatized into the nursery pond, stocked (0.50 $\pm$ 0.11 g), and reared (10 m^{-2}) into the ponds with three feeding modes. The salinity of ponds was 5.47$\pm$ 0.53 ppt. The animals were fed 100%

(a) (b)

Fig. 1. Design of aquamimicry pond with central pit (a) and experimental pond (b).

Table 1
The comparative view of existing traditional ponds and aquamimicry pond.

Features	Traditional pond	Aquamimicry pond
Central pit	No	Yes
Suction pump	No	Yes
Aerator	No	Yes
Fence	Yes	Yes
Reservoir	No	No

Table 2
Different steps of natural food production during pond preparation of the aquamimicry treatment ponds.

Days	Materials Applied/acre	Purposes
1st - 5th	Bleaching powder (6 ppm)	Disinfection of water
6th - 8th	6 kg LFRB	Production of natural food
9th	4 L water probiotics (super PS)	Control the growth of harmful bacteria
10th	5 kg of LFRB	Support the plankton production
11th	6 L of soil probiotics (Superbiotic)	Control the growth of harmful bacteria
13th	7 kg of minerals	Production of natural food
14th	5 kg of LFRB	For the copepods production
15th	4 L water probiotic	Control the growth of harmful bacteria

Table 3
Experimental design of shrimp (*P. monodon*) culture.

Treatments	Pond design	Feed application
T_0	Improved traditional	100% Commercial Feed
T_1	Aquamimicry	10% LFRB + 90% Commercial Feed
T_2	Aquamimicry	30% LFRB + 70% Commercial Feed

commercial feed (CF), 10% LFRB + 90% CF, and 30% LFRB + 70% CF in the control (T_0), Treatment 1 (T_1) and Treatment 2 (T_2), respectively. According to the packaging label of that commercial feed, the nutritional composition (dry matter basis) was: Moisture (Max):11%, Crude Protein (Min):29%, Fat (Min): 7%, Carbohydrate (Max):22%, Fiber (Max): 3.5%, Ash (Max): 10%, Calcium (Max): 2.5%, and Phosphorus (Min): 2%. The feed was given 10%, 5%, and 4% of body weight for the weight range of 1–5, 5–10, and 10–30 g, respectively. Ten animals were sampled and weighed weekly from each experimental unit, and the daily amount of feed was adjusted accordingly.

2.5. Monitoring of water quality parameters

Temperature was monitored twice daily using a digital thermometer (Digi-Thermo WT-2, King Till, Suzhou, China) at different locations in the pond. Transparency was measured at weekly intervals using a Secci disc. A digital DO meter (HACH, USA) was used to determine the dissolved oxygen content of the water and measured twice daily in morning and afternoon. The pH and salinity were measured using a digital pH meter (HACH) and a refractometer, respectively. pH was recorded daily in morning and salinity was recorded on weekly basis in morning. Ammonia (mg L^{-1}) was determined by a test kit (HANNA) and measured daily in the morning shortly after feeding. The plankton quantity in the cultured ponds was analyzed fortnightly. A 1 mL sample was counted in Sedgewick Rafter (SR) cell chambers under a 100× microscope, and cell density was computed according to Stirling (1985) using the following formula:

$$N = \frac{A \times 1000 \times C}{V \times F \times L}$$

where N=Number of plankton cells or units L^{-1} of original water
A=Total number of plankton counted
C=Volume of final concentration of the sample (mL)
V=Volume of a field (1 mL)
F=Number of the field counted
L= Volume of original water (L)

2.6. Growth parameters

The shrimp growth parameters were recorded weekly and at the end of the experiment. The shrimps were starved for approximately 12 h and weighed separately using an electric balance. After 90 days of rearing, specific growth rate (SGR), average weight gain (AWG), and survival rate (%) were calculated using the following equation:

$$SGR \quad \left(\%day^{-1}\right) = \frac{Ln \quad final \quad weight \quad (g) - Ln \quad initial \quad weight(g)}{Period \quad in \quad days} \times 100$$

$$AGR = \frac{Mean \quad final \quad weight \quad (g) - Mean \quad initial \quad weight(g)}{Period \quad in \quad days}$$

$$Survival \quad rate(\%) = \frac{No.of \quad harvested \quad shrimp}{No.of \quad initial \quad stocked \quad shrimp} \times 100$$

2.7. Microbial analysis

Microbial analyses of samples collected from water, soil, and shrimp culture ponds were performed separately to isolate and characterize the microbes fortnightly. Specific media were used to culture *Vibrio* species for identification. After enrichment of the sample in peptone-enriched alkaline water, serial dilutions and plating were performed. Thiosulfate citrate bile salts sucrose agar (TCBS, Difco, Detroit, MI, USA), which is the selective medium for the *Vibrio* sp, was used was used. The subculture was streaked from the enrichment medium onto selective agar (TCBS). Culture plates were incubated for 24 h at 35°C. Bacterial species were identified by observing the colony morphology, according to Lee et al. (1978), Alsina and Blanch (1994), Davis et al. (1981), and Lavilla-Pitogo et al. (1998).

2.8. Hemolytic test

A hemolysis test was performed for four types of samples: water, soil, hepatopancreas, and shrimp muscle, according to Jonas and Broad (1972). Briefly, blood agar (40.0 g) was suspended in 1000 mL of distilled water and heated. Subsequently, sterilization was performed by autoclaving at 15 lb pressure and 121°C for 15 min. was After it cooled to 50°C, 5% v/v sterile defibrinated blood was added aseptically. The sample was mixed thoroughly and sterile Petri plates were prepared. The sample was then streaked onto the plates and incubated at 37°C overnight.

2.9. Total hemocyte count (THC)

Total hemocyte count (THC) analysis was conducted on twenty shrimp from each treatment group over a period spanning from the 7th week to the 11th week of the experiment before the mortality of shrimp of control pond. This timeframe was selected to facilitate the ease of hemocyte collection, aligning with the weekly weight measurement schedule implemented from the 7th week onwards. A hemolymph sample of 0.5 mL was withdrawn from the base of the 3rd walking leg of each shrimp using a syringe containing 1.5 mL anticoagulant. After collecting the hemolymph, the hemocytes were counted using a hemocytometer. The number of blood cells per volume was calculated (total hemocytes per cubic millimeter) according to Song and Hsieh (1994).

2.10. Histological analysis

The hepatopancreas was removed and preserved in 10% buffered formalin until histological analysis. Histological samples were transversally sectioned, dehydrated, and embedded in paraffin, following standard procedures (Lightner, 1996) and then observed under a microscope.

2.11. Statistical analysis

Data were analyzed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Data were analyzed for normality using probability plots and homogeneity of variances using Levene's test. ANOVA analysis was used to determine the significance of each parameter among the different treatments. Differences between means were tested using Tukey's test at a 5% probability level.

3. Results

3.1. Water quality parameters

The average mean values of physicochemical parameters, such as temperature, dissolved oxygen (DO), pH, salinity, alkalinity, ammonia, and transparency, during the experimental period under different treatments, are presented in Table 4. There were no significant differences ($P > 0.05$) in the temperature, DO, or alkalinity among the treatments during the experimental period. Among the treatments, the aquamimicry treatment ponds had significantly lower levels ($P< 0.05$) of transparency, pH, and salinity than the control ponds, with T_2 exhibiting the lowest values. No ammonia toxicity was observed in the culture ponds during the experimental period.

The average plankton quantity in each treatment pond during the experimental period is shown in Fig. 2. Plankton counts were significantly higher in the T_1 and T_2 ponds than in the control culture ponds. In total, 217.04 mm of rainfall was recorded between the 2nd and 4th weeks of the experimental period (Satkhira Climate Weather Averages, 2022), and the quantity of plankton decreased; however, the portion in the T_1 and T_2 ponds remained significantly higher than in the control ponds (T_0). The average amount of plankton was the highest (9.75×10^3 cells L^{-1}) in T_2 and the lowest (4.5×10^3 cells L^{-1}) in the control ponds where no aquamimicry treatment was used.

3.2. Growth performance and survival rate

The AGR (g day^{-1}) and SGR (% day^{-1}) of black tiger shrimp (*P. monodon*) in the different treatments are listed in Table 5. The weekly weight gain and survival rates (%) of the treatments are shown in Figs. 3 and 4. The highest and lowest SGRs were observed in the treatment T_2 ($3.07 \pm 0.30\%$ day^{-1}) and control T_0 ($1.93 \pm 0.34\%$ day^{-1}). There was a significant difference ($P<0.05$) in SGR (% day^{-1}) between the aquamimicry treatments (T_1 and T_2) and the control (T_0). In the 11th week, the average weight gain of shrimp was highest in T_2 (19 ± 2.02 g) and lowest in T_0 (10 ± 1.5 g). Finally, the average weight of shrimp was significantly higher in T_2 (33 ± 1.12 g), where 70% CF + 30% LFRB feeding was supplied than the average weight of shrimp in T_1 (27 ± 0.94 g), where 90% CF + 10% LFRB was supplied. The average daily weight gain of the shrimp was 0.05 ± 0.00, 0.34 ± 0.05, and 0.47 ± 0.06 g day^{-1} in the treatments T_0, T_1, and T_2, respectively.

The weekly survival rate (Fig. 2) indicated that the shrimp had a higher survival rate in aquamimicry pond T_2 than in ponds T_1 and T_0. After the 11th week of stocking, almost all shrimp died in the control pond (T_0). At the end of the experiment, the survival rate of shrimp was significantly higher in T_2 (55%) and T_1 (45%) than in T_0 (1%).

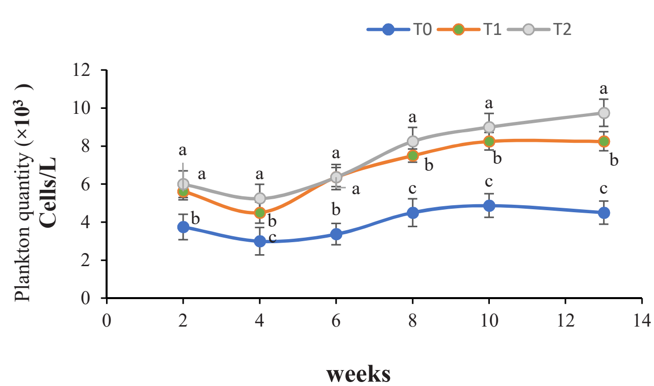


Fig. 2. Quantity of plankton in control and aquamimicry ponds stocked with black tiger shrimp (*P. monodon*).

Table 4
Average water quality parameters in control and aquamimicry ponds stocked with black tiger shrimp (*P. monodon*) reared for 90 days. Values are Mean ± Standard Deviation; n = 3. Mean values with different superscript letters in the same column are significantly different ($P < 0.05$).

Treatment	pH	DO (ppm)	Alkalinity	NH_3 (ppm)	Temperature (°C)	Salinity (ppt)	Transparency (cm)
T_0	8.11 ± 0.14^a	5.74 ± 0.4^a	78.75 ± 3.56^a	0.0	33.3 ± 1.51^a	5.67 ± 0.37^a	54 ± 4.07^a
T_1	7.81 ± 0.07^b	5.88 ± 0.3^a	74.47 ± 2.94^a	0.0	33 ± 3.47^a	5.37 ± 0.50^b	48 ± 3.05^{ab}
T_2	7.79 ± 0.08^b	6.01 ± 0.5^a	74.37 ± 3.07^a	0.0	31.8 ± 2.77^a	5.39 ± 0.72^b	45 ± 3.58^b

Table 5
Average growth rate (g day^{-1}) and specific growth rate (% day^{-1}) of black tiger shrimp (*P. monodon*) reared in control and aquamimicry ponds with different feeding types for 90 days. Values are Mean ± Standard Deviation; n = 3.

Treatments	T_0	T_1	T_2
SGR (%day^{-1})	1.93 ± 0.34^{c}	2.1 ± 0.11^{b}	3.07 ± 0.30^{a}
AGR (g day^{-1})	0.05 ± 0.00^{c}	0.34 ± 0.05^{b}	0.47 ± 0.06^{a}

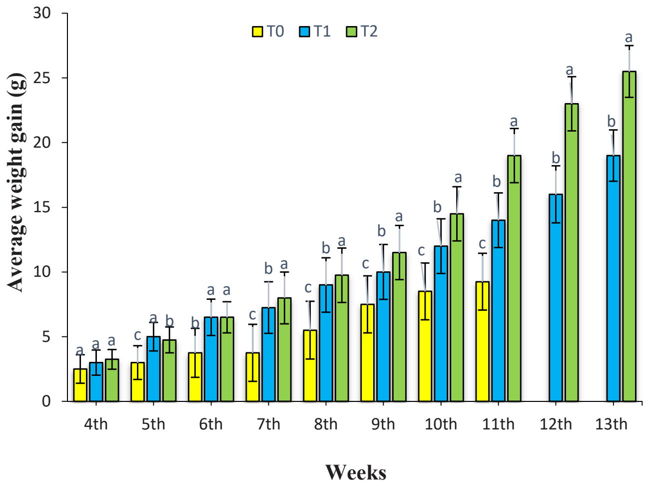


Fig. 3. Average weekly weight gain of black tiger shrimp (*P. monodon*) reared in control and aquamimicry ponds with different feeding types for 90 days (Sampling was started from the 4th week of stocking). Values are Mean ± Standard Deviation; n = 3. Mean values with different superscript letters are significantly different ($P < 0.05$).

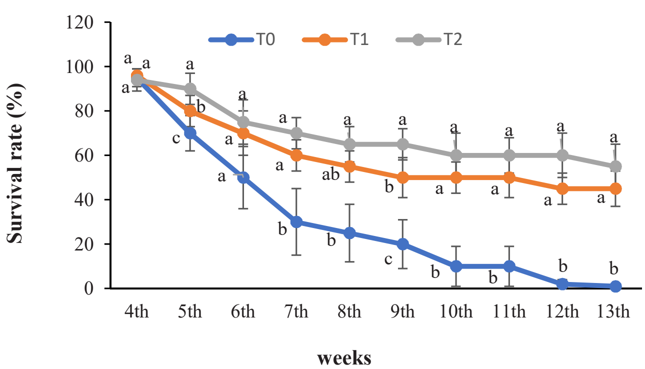


Fig. 4. Weekly survival rate (%) of black tiger shrimp (*P. monodon*) reared in control and aquamimicry ponds with different feeding types for 90 days. Values are Mean ± Standard Deviation; n = 3. Mean values with different superscript letters are significantly different ($P < 0.05$).

3.3. Microbial status

According to the microbial analysis, several species of *Vibrio* and *Proteus* were identified in the water, soil, hepatopancreas, and muscle of the shrimp (Table 6). The water and hepatopancreas of shrimp in the control pond contained the pathogenic bacterium *V. parahaemolyticus* (Fig. 5). In contrast, the pathogenic strain *V. parahaemolyticus* was not found in the aquamimicry treatment ponds.

3.4. Hemolytic nature

Some notable differences were observed in the hemolytic nature of the bacteria among the treatments (Table 7). Significantly, the bacteria isolated from the shrimp muscle and hepatopancreas in the control ponds showed complete hemolysis. In contrast, the bacteria collected from the aquamimicry treatments (T_1 and T_2) were either partially or non-hemolytic. In the case of water and soil, the bacteria isolated from treatment T_1 showed partial hemolysis, and T_2 showed non-hemolysis, whereas the bacteria collected from T_0 showed complete or partial hemolysis (Fig. 6).

3.5. Health status

The THC from the shrimp in T_1 and T_2 was higher than that of the shrimp in the control ponds (T_0) (Table 8). The THC from the shrimp of T_2 with the aquamimicry pond design and 30% LFRB was significantly higher ($P< 0.05$) than in the other two treatments.

Histology of the shrimp hepatopancreas showed the standard and well-developed shape of the hepatopancreatic tubules in T_1 and T_2, where the shrimp in T_2 had the most regular form. However, at T_0, an irregular and undeveloped hepatopancreatic tubule was observed (Fig. 7).

4. Discussion

The concept of aquamimicry has been established and adopted in shrimp culture to provide natural conditions for shrimp production (Khanjani et al., 2023). The success of this strategy involves lowering the feed conversion ratio, preventing disease outbreaks, reducing water exchange, and improving production quality (Zeng et al., 2020). Many studies have reported that the application of fermented carbohydrates in ponds increases plankton density, mainly of copepods, by utilizing heterotrophic fauna that mimic the natural environment of organisms in confined water conditions (Deepak et al., 2020; Romano and Kumar, 2017). Damayanti et al. (2020) reported that live food (*Moina macrocopa*) production with fermented rice bran suspensions was higher than that without fermentation. Similar results were found in the present study, with a significantly higher plankton abundance in the aquamimicry treatment ponds (T_1 and T_2) than in the control pond (T_0). LFRB are supplemented with probiotics in aquamimicry-treated ponds that can boast abundant natural food (phytoplankton and zooplankton) and create natural pond conditions. All ponds during the experimental period had suitable water quality parameters for black tiger shrimp (Amalia et al., 2022; Mustafa et al., 2022; Arifin et al., 2007; Lazur, 2007). Although salinity was slightly low but optimum for *Penaeus monodon* culture (Rahi et al., 2021).Therefore, including LFRB did not alter the chemistry of the ponds. One remarkable feature of the aquamimicry treatment ponds was that they were significantly less transparent, which might be due to the more natural feeds than the traditional pond in this experiment. Literature supports the notion that the presence of live feeds can influence water clarity (Das et al., 2012) and the turbidity level of the water in aquamimicry system determines the appropriate inclusion of FRB (Khanjani et al., 2022).

In this study, pond design and feeding type significantly affected the average weight gains, SGR, and AGR of shrimp. The shrimp stock in the control ponds, where traditional culture techniques were used, crashed within 11 weeks of stocking. In contrast, shrimp growth and survival rates were significantly higher in aquaculture-treated ponds than in control ponds. Among the aquamimicry ponds, shrimp in T_2, with 30% LFRB, had a significantly higher growth performance than the shrimp of T_1, with 10% LFRB. The average growth rate (0.34 g day^{-1}) in the semi-intensive culture system found by Janakiram et al. (2011) complied with the present study. In the case of *Litopenaeus vannamei*, the highest SGR (4.43% day^{-1}) in an outdoor zero-water-exchange culture system over an eight weeks experimental period, found by Tacon et al. (2002), was very similar to the present findings. The enhancement of growth indices in shrimp in aquamimicry-treated ponds may be linked to the inclusion of LFRB. The increased nutritional availability of LFRB and the reduction in anti-nutritional factors due to fermentation may be responsible

Table 6
Major microbial observation in control and aquamimicry ponds stock with black tiger shrimp (*P. monodon*).

Treatments	Ponds	Types of sample	Colony colour	Colony size(mm)	Remarks
T_1	T_1R_1	Water	Yellow	2–3	*Vibrio fluvialis*
		Soil	Yellow	2–3	*V. fluvialis*
		Hepatopancreas	Yellow	2–3	*V. fluvialis*
		Muscle	Yellow	2–3	*V. fluvialis*
	T_1R_2	Water	Yellow & Green	2–3	*V. fluvialis, Proteus sp*
		Soil	Yellow	2–3,3–4	*V. fluvialis, V. metschenikovii*
		Hepatopancreas	Yellow & Green	2–3	*V. fluvialis, Proteus sp.*
		Muscle	Yellow	2–3	*V. fluvialis*
	T_1R_3	Water	Yellow	2–3	*Vibrio fluvialis*
		Soil	Yellow	2–3	*V. fluvialis*
		Hepatopancreas	Yellow	2–3	*V. fluvialis*
		Muscle	Yellow	2–3	*V. fluvialis*
T_2	T_2R_1	Water	Yellow	2–3	*V. fluvialis*
		Soil	Yellow	2–3	*V. fluvialis*
		Hepatopancreas	Yellow & Green	2–3	*V. fluvialis, Proteus sp.*
		Muscle	Yellow	2–3	*V. fluvialis*
	T_2R_2	Water	Yellow	2–3	*V. fluvialis*
		Soil	Yellow	2–3	*V. fluvialis*
		Hepatopancreas	Yellow & Green	2–3	*V. fluvialis, Proteus sp.*
		Muscle	Yellow & Green	2–3	*V. fluvialis, Proteus sp.*
	T_1R_3	Water	Yellow	2–3	*V. fluvialis*
		Soil	Yellow	2–3	*V. fluvialis*
		Hepatopancreas	Yellow & Green	2–3	*V. fluvialis, Proteus sp.*
		Muscle	Yellow & Green	2–3	*V. fluvialis*
T_0	T_0R_1	Water	Blue green	3–5	*V. parahaemolyticus*
		Soil	Yellow, Blue green	2–3,3–5	*V.fluvialis, V.parahaemolyticus*
		Hepatopancreas	Yellow & Green	2–3	*V. fluvialis, Proteus sp.*
		Muscle	Yellow & Green	2–3	*V. fluvialis, Proteus sp.*
	T_0R_2	Water	Yellow, Blue green	2–3, 3–5	*V. fluvialis, V.parahaemolyticus*
		Soil	Yellow	2–3	*V. fluvialis*
		Hepatopancreas	Yellow, Blue green	2–3, 3–5	*V. fluvialis, V. parahaemolyticus*
		Muscle	Yellow	2–3	*V. fluvialis*
	T_0R_3	Water	Yellow, Blue green	3–5	*V.parahaemolyticus*
		Soil	Yellow	2–3	*V. fluvialis*
		Hepatopancreas	Yellow, Blue green	3–5	*V. parahaemolyticus*
		Muscle	Yellow	2–3	*V. fluvialis*

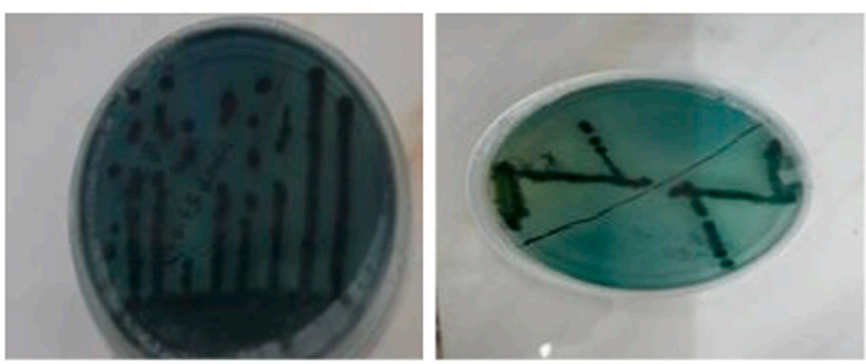
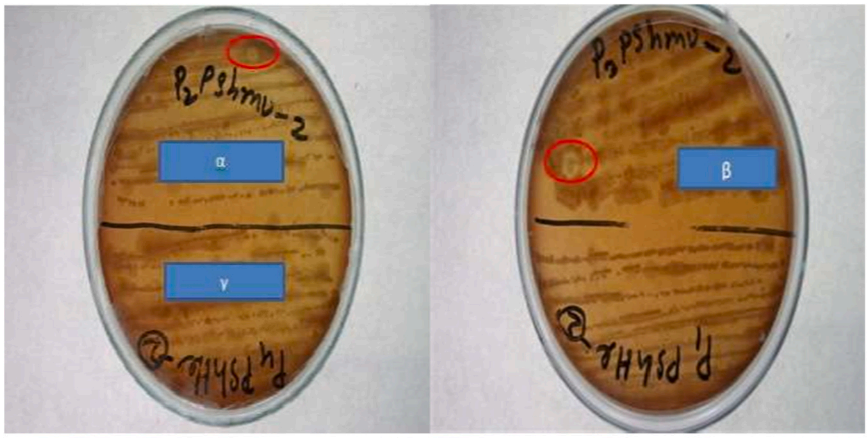


Fig. 5. Colonies of suspected *V. parahaemolyicus* found in the control ponds.

Table 7
Hemolytic nature of the bacteria isolated from water, soil, hepatopancreas and muscle of black tiger shrimp (*P. monodon*) in different treatments. α-Alpha= incomplete hemolysis and partial hemolysis, β-Beta= complete hemolysis & γ-Gamma = non-hemolytic.

Treatments	Water	Soil	Hepatopancreas	Muscle
T_1R_1	α	α	Γ	α
T_1R_2	α	α	A	α
T_1R_3	α	α	A	α
T_2R_1	γ	γ	Γ	α
T_2R_2	γ	γ	Γ	α
T_2R_3	γ	γ	Γ	α
T_0R_1	β	β	B	β
T_0R_2	α	β	B	β
T_0R_3	β	β	B	β

for the enhanced growth performance (Refstie et al., 2005; Zeng et al., 2020; Cheng et al., 2017; Qiu and Davis,2018). According to Romano et al. (2018), fermented rice bran improved the water conditions, growth parameters, feeding efficiencies, and biochemical composition of African catfish (*Clarias gariepinus*) juveniles in the biofloc culture system, which is quite obvious in this study. In addition, the application of LFRB enhances live food production in aquamimicry, which are abundant in long-chain polyunsaturated fatty acids (LC-PUFA), minerals, trace elements, pigments, and a variety of free amino acids such as taurine, which may result in improved feed efficiency and enhanced immune competence in shrimp (Khanjani et al., 2022). Both beneficial microorganisms and natural plankton produced in aquamimicry ponds may act as good sources of supplementary nutrients for fish and shrimp and improve biological productivity in ponds (Wasielesky et al., 2006; Samocha et al., 2007; Nisar et al., 2021; Catalani et al., 2023; Khanjani



(a) (b)

Fig. 6. Hemolytic nature of bacteria isolated from water, soil, hepatopancreas and muscle of black tiger shrimp reared in different treatments. α-Alpha= incomplete hemolysis (red circle in picture **a**) and partial hemolysis, β-Beta= complete hemolysis (red circle in picture **b)** & γ-Gamma = non-hemolytic.

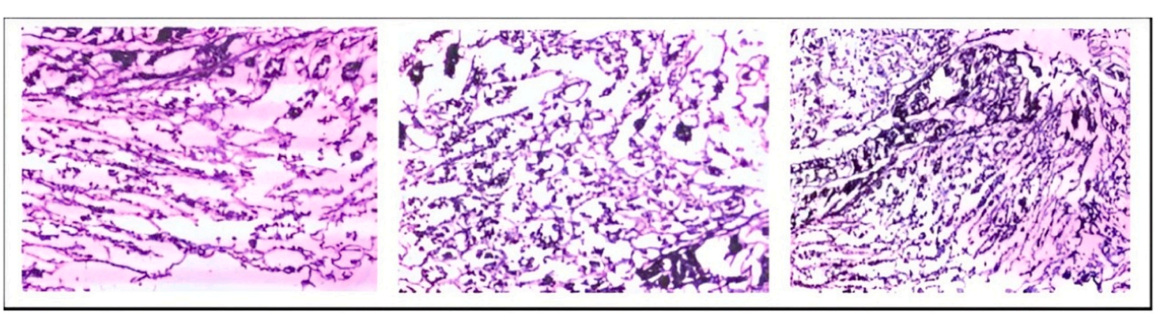
Table 8
Total haemocyte count of shrimp reared in control and aqumimicry ponds with different feeding types.

Parameters	T_0 ($\times 10^3$ cells mL^{-1})	T_1 ($\times 10^3$ cells mL^{-1})	T_2 ($\times 10^3$cells mL^{-1})
Total Haemocyte Count (THC)	2.5 ± 0.12^b	2.9 ± 0.1^b	3.6 ± 0.08^a

et al., 2023).

According to the results of this study, possibly the pathogenic strain of *Vibrio parahaemolyticus* infected shrimp in the control ponds with traditional systems, whereas the pathogen was absent in the aquamimicry treatment ponds. A hemolytic test confirmed the presence of pathogenic strains. Beta hemolysis reflects the pathogenicity of microorganisms (Sum et al., 2017). The beta-hemolytic nature of the bacteria isolated from the soil, water, and shrimp samples of the control indicated the presence of a pathogenic strain. Mortality in the control pond after the 11th week of stocking could be due to pathogenic bacteria, possibly *V. parahaemolyticus*, which is responsible for early mortality syndrome (EMS) in *P. monodon*. Disease infestation caused a sudden mortality up to 100% by *Vibrio parahaemolyticus* (Hong To et al.,2020 & Intriago et al., 2023). Several researchers reported that aquamimicry and biofloc technology can effectively reduce the levels of total ammonia nitrogen, nitrite, and harmful opportunistic bacteria, including *Vibrio* species, in aquaculture systems (Brito et al., 2016; Hostins et al., 2019; Khanjani et al., 2022). Moreover, probiotics which are used in this experiment for pond preparation and Liquid fermented rice bran preparation (*Rhodobacter* sp, *Bacillus subtilis, Bacillus amyloliquefaciens*) have the capability to inhibit pathogenic *Vibrio parahaemolyticus* (Proespraiwong et al., 2023; Kewcharoen and Srisapoome, 2019). The survival rate, growth, and postmortem malpigmentation of fish and shrimp are notably enhanced by an increased density of plankton, particularly copepods found in several studies (Shields et al., 1999; Rajkumar & Vasagam, 2006; Santhanam et al., 2020; Abbaszadeh et al., 2022; Liñan et al.,2020 and Nisar et al., 2021). Shrimp mortality usually occurs when the bottom of a shrimp pond is polluted and deteriorates beyond the remediation capacity of the microbes (Boyd and Musig, 1992). The presence of excess feed in shrimp ponds leads to the accumulation of unwanted organic waste and harmful gases in bottom soils. Sudden mortality in the control ponds could have occurred due to these causes. In contrast, in the aquamimicry treatment pond, the excess waste accumulated in the central pit was periodically cleaned using a suction pump. Furthermore, in aquamimicry culture systems, in situ waste assimilation boosts beneficial heterotrophic microorganisms that decompose organic matter, thus improving the quality of culture water (Maicá et al., 2014; Vijayan et al., 2019). The better biochemical composition of the plankton and healthy environment of the aquamimicry ponds could support a better survival rate in the aquamimicry-treated ponds in the present study.

The highest and lowest total hemocyte counts (THC) were observed at T_2 and T_0, respectively. Higher numbers of hemocytes are associated with increased resistance to pathogenic bacteria. To a certain extent, THC affects the immune response in shrimp (Ji et al., 2009). Hemocytes constitute the first line of internal defense against invaders and are crucial for the immune reactions of crustaceans (Bachère et al., 1995; Shiu-Nan, 1992). Le Moullac et al. (1998) reported that *Penaeus*



(a) (b) (c)

Fig. 7. Representative photomicrographs of the hepatopancreas of shrimp of different treatments [(a) T_0; (b) T_1; (c) T_2].

styliriostris with a low THC was more susceptible to infections with *Vibrio* species. The higher survivability of shrimp in T_1 and T_2 could be associated with higher THC. Therefore, this study indicated that aquamimicry treatment with LFRB has a positive effect on the immune response in shrimp.

The histology of the hepatopancreas describes the health condition of the shrimp. The hepatopancreas (HP) is an indicator of the shrimp body that can be used to identify health conditions (Manan et al., 2015). The immune system protects the body against microorganisms, removes tissues damaged by trauma, and eliminates malignant growth. Bautista et al. (1994) reported that the hepatopancreas and liver are highly sensitive to different diets and waterborne pollutants; thus, they are often used to monitor the effects of various toxicants. Regular and well-developed hepatopancreatic tubules indicated that the shrimp were healthy. In EMS infect infected and affected shrimp usually showed an irregular, small, swollen, and discolored hepatopancreas (Lightner et al., 1996). The hepatopancreas of healthy shrimps usually exhibit a well-organized glandular tubular structure. The loss of essential biological functions in the hepatopancreas ultimately affects the survival and growth of *P. monodon* (Kumar et al., 2010). The regular and well-developed hepatopancreatic tubules of shrimp in the aquamimicry treatment ponds, especially in the T_2 treatment, revealed their healthy condition compared to the irregular and undeveloped hepatopancreatic tubules of shrimp in the control pond.

5. Conclusion

This study thoroughly explored the cumulative outcomes, revealing favorable results for black tiger shrimp's growth performance, immunity, and survival rates within the aquamimicry framework. Aquamimicry treatment ponds, especially T_2 with 70% CF + 30% LFRB, exhibited the best growth performance, survival rate, and immunological parameters for *P. monodon* compared to the control treatment. The microbial analysis, hemocyte count, and histological analysis confirmed that better-quality shrimp were found in the aquamimicry treatment ponds than in the traditional ponds. Therefore, this technology has great potential to improve overall growth, survival, and immunity in black tiger shrimp (*P. monodon*) production. The findings of this study support the implementation of aquamimicry as a means of mitigating feed expenses and effectively managing the emergence of diseases in black tiger shrimp.

CRediT authorship contribution statement

Mousumi Das: Writing – review & editing, Resources, Formal analysis. **Md. Shoebul Islam:** Writing – original draft, Methodology, Formal analysis, Data curation. **Jong Min Lee:** Writing – review & editing. **Koushik Chakroborty:** Writing – review & editing, Data curation. **S. M. Rafiquzzaman:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Md.Rabiul Islam:** Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. S.M. Rafiquzzaman reports financial support was provided by Krishi Gobeshona Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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