



## Farm-scale evaluation of a fermented probiotic–rice bran formulation on pond conditions, gut bacteria, immunity, and production performance of *Penaeus monodon* in semi-intensive shrimp ponds

Saroj Kumar Mistry<sup>1,2</sup> • Syed Hafizur Rahman<sup>1</sup> • Md. Golam Sarower<sup>3</sup>

<sup>1</sup> Department of Environmental Sciences, Jahangirnagar University, Dhaka 1342, Bangladesh

<sup>2</sup> Department of Fisheries, Ministry of Fisheries and Livestock, Dhaka 1205, Bangladesh

<sup>3</sup> Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna 9260, Bangladesh

### Correspondence

Md. Golam Sarower; Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna 9260, Bangladesh  
✉ [sarower@fmrt.ku.ac.bd](mailto:sarower@fmrt.ku.ac.bd)

### Manuscript history

Received 6 September 2026 | Accepted 5 October 2026 | Published online 8 October 2026

### Citation

Mistry SK, Rahman SH, Sarower MG (2027) Farm-scale evaluation of a fermented probiotic–rice bran formulation on pond conditions, gut bacteria, immunity, and production performance of *Penaeus monodon* in semi-intensive shrimp ponds. Journal of Fisheries 15(1): 151207. DOI: 10.17017/j.fish.1510

### Abstract

Sustainable shrimp production depended on maintaining suitable pond environments, microbial balance, and efficient production practices. However, the effectiveness of microbial management strategies during complete production cycles under farm conditions has remained insufficiently documented because previous studies have mainly focused on laboratory experiments or short-term trials. This study investigated probiotic supplementation and a fermented probiotic–rice bran formulation in semi-intensive *Penaeus monodon* culture systems in coastal Bangladesh. The influence of these treatments on pond characteristics, gut bacterial populations, immune responses, growth performance, and economic returns was evaluated. The 152-day trial was conducted in nine ponds assigned to control, probiotic, and fermented probiotic–rice bran formulation groups, with three replicate ponds per treatment. The fermented formulation treatment resulted in lower sediment organic matter accumulation, higher dissolved oxygen and alkalinity, and reduced diurnal pH fluctuations compared with the other treatments. At harvest, shrimp receiving the fermented formulation showed higher presumptive *Bacillus* spp. and lower presumptive *Vibrio* spp. bacterial abundance in the gut, along with higher haemocyte count, lysozyme activity, prophenoloxidase activity, and superoxide dismutase activity. The treatment also resulted in the lowest feed conversion ratio, greatest final body weight, and highest production (6111.67 kg ha<sup>-1</sup>). Despite higher production costs, the fermented formulation generated the highest net profit and benefit–cost ratio (1.87). Overall, the fermented formulation enhanced pond conditions, gut bacterial abundance patterns, physiological responses, feed efficiency, production performance, and profitability. The findings indicated that this approach improved measured physiological responses, production performance, and farm profitability under semi-intensive coastal farming conditions.

**Keywords:** innate immunity; *Penaeus monodon*; pond water quality; production efficiency; sediment quality; synbiotic

## 1 | INTRODUCTION

Black tiger shrimp (*Penaeus monodon*) farming is an important part of coastal aquaculture in Asia, supporting seafood production, export earnings, employment, and the livelihoods of coastal communities (Ahmed and Diana 2015; Abdullah *et al.* 2017). In Bangladesh, *P. monodon* is one of the major cultured shrimp species, with production concentrated along the southwestern coast, where shrimp farming contributes to household income and local economies (Azad *et al.* 2009; Rahman *et al.* 2014; Abdullah *et al.* 2017). Increasing market demand has encouraged the expansion of semi-intensive farming systems with higher stocking densities and feed inputs (Alam 2023). As shrimp farming becomes more intensive, higher stocking densities and increased feed use can result in greater accumulation of organic matter and nutrients in ponds. Uneaten feed, faecal matter, and plankton residues contribute to this organic load. Their decomposition increases oxygen demand and can affect sediment and water quality, which may eventually influence shrimp growth and survival (Chen and Lei 1990; Wahab *et al.* 2003; Han *et al.* 2017; Lin *et al.* 2024).

In coastal Bangladesh, seasonal variations in rainfall, evaporation, and salinity further contribute to fluctuations in pond conditions and shrimp production (Ahmed and Diana 2015). Pond environmental conditions are closely connected with microbial communities in the culture environment and the gut microbiota of shrimp. These gut-associated microorganisms play important roles in digestion, nutrient metabolism, and immune development (Rungrassamee *et al.* 2013, 2014; Rajeev *et al.* 2021). *Vibrio* spp. occur naturally in aquatic environments and are commonly found in shrimp culture systems. However, under stressful environmental conditions or when the microbial balance is disturbed, some *Vibrio* spp. populations may increase and pose a risk to shrimp health (Cornejo-Granados *et al.* 2017). Therefore, monitoring presumptive *Vibrio* spp. bacterial abundance can provide useful information on microbial changes associated with pond conditions and shrimp health.

Environmental stress and microbial imbalance may also influence shrimp physiological stability and innate immune responses. Total haemocyte count, lysozyme activity, and the prophenoloxidase system are widely used indicators of shrimp cellular and humoral defence mechanisms, whereas superoxide dismutase contributes to antioxidant protection against oxidative stress (Amparyup *et al.* 2013; Duan *et al.* 2015). In addition, inappropriate chemical use in aquaculture may disturb beneficial microbial communities and create environmental concerns (Cabello 2006). Together, these concerns have increased interest in microbial-based approaches to improve pond conditions and support shrimp health.

Probiotics and synbiotics have been widely studied as approaches for managing microbial communities in

shrimp farming. Probiotics may influence microbial balance through competition with other microorganisms, production of inhibitory compounds, and changes in nutrient cycling, while synbiotics combine beneficial microorganisms with suitable substrates to support microbial activity and host health (Verschuere *et al.* 2000; Swanson *et al.* 2020; Mathan Muthu *et al.* 2024). Fermented organic substrates such as rice bran may also provide nutrients that support beneficial microorganisms during preparation and application. Previous studies have reported improvements in shrimp growth, bacterial composition, immune responses, and disease resistance following microbial supplementation (Widanarni *et al.* 2013; Hasyimi *et al.* 2020; Rohani *et al.* 2022). Recent work has also shown the potential of synbiotic approaches to improve resistance to bacterial challenges in penaeid shrimp (Chin *et al.* 2024). However, much of the available evidence has been generated from laboratory experiments, short-term feeding trials, biofloc systems, or pathogen-challenge studies. Such experimental conditions may not fully represent responses under commercial pond farming conditions.

Evidence from complete farm-scale production cycles integrating environmental, microbial, physiological, and economic responses remains limited, particularly for semi-intensive *P. monodon* culture in coastal Bangladesh. Previous studies have rarely evaluated changes in pond soil and water quality, gut bacterial populations, immune responses, growth performance, and economic outcomes within the same farming system (Goh *et al.* 2022; Khanjani *et al.* 2024). Furthermore, whether fermented probiotic formulations provide additional advantages over conventional probiotic supplementation under practical pond conditions remains insufficiently understood.

Therefore, farm-scale evaluation is needed to determine whether microbial interventions can provide consistent benefits beyond controlled experimental environments. In this study, control, probiotic, and fermented probiotic–rice bran formulation treatments were compared under semi-intensive *P. monodon* farming conditions in coastal Bangladesh. The effects of these treatments on pond environmental conditions, gut bacterial populations, physiological responses, growth performance, and economic returns were evaluated. The relationships among environmental, microbial, immune, and production responses were also examined. We hypothesized that microbial supplementation would improve pond conditions and shrimp performance, and that the fermented probiotic–rice bran formulation would provide greater benefits than probiotic supplementation alone.

## 2 | METHODOLOGY

### 2.1 Study site and experimental design

The study was conducted in a semi-intensive shrimp farm

with similar physical characteristics, including shape, size, and water depth, in Dumuria Upazila, Khulna District, southwestern Bangladesh (22°46'53.9"N 89°27'53.6"E). Three treatments were applied: a control treatment without microbial supplementation (SIC), a probiotic treatment (SIP), and a fermented probiotic–rice bran formulation treatment (SIS). The nine ponds were randomly assigned to the three treatments, with three independent replicate ponds allocated to each treatment. Each pond was considered an experimental unit to prevent pseudoreplication. All ponds received water from the same source and were managed under identical conditions, including the use of the same post-larvae source, commercial feed, mineral supplementation, aeration, and routine pond-management practices. The primary difference among treatments was the application of different microbial formulations.

## 2.2 Pond preparation and management

Before stocking, the ponds were drained, exposed to sunlight for drying, and repaired where necessary. Agricultural lime (CaCO<sub>3</sub>) was applied at a rate of 247 kg ha<sup>-1</sup> according to initial soil pH and standard pond-management recommendations (Boyd and Tucker 1998). Pond water was supplied from a nearby river. Before entering the ponds, the water was filtered through a 200-µm mesh and disinfected with bleaching powder at 60 mg L<sup>-1</sup> with 35% active chlorine. Net fencing was installed around each pond for biosecurity. Biosecurity measures were followed throughout the culture period. Access to the culture area was restricted, and the farm was managed by trained personnel following routine biosecurity practices. Specific pathogen-free (SPF) post-larvae were used for stocking. These measures helped reduce the risk of pathogen entry and spread and the occurrence of disease in the experimental ponds.

Water depth was maintained at approximately 1.50 m through controlled water exchange. Each pond was equipped with two 2.0-hp paddle-wheel aerators. Aeration was initially provided for approximately 4 h day<sup>-1</sup> and gradually increased to 18 h day<sup>-1</sup> toward the end of the culture period as shrimp biomass increased. The same aeration schedule was followed in all treatments.

## 2.3 Shrimp stocking and feeding

All ponds were stocked with specific-pathogen-free post-larvae (PL) of *P. monodon* obtained from a certified commercial hatchery. PL15 shrimp from the same batch were stocked at a density of 14 post-larvae m<sup>-2</sup> with an initial mean body weight of approximately 0.001 g individual<sup>-1</sup>. Stocking was carried out on 16 May 2024 and the shrimp were harvested on 15 October 2024, resulting in a 152-day culture period.

Shrimp were fed a commercial diet (Avanti Shrimp Feed, India) containing 42% crude protein four times dai-

ly. Feed ration was adjusted according to estimated biomass and routine farm-feeding practices. MagKcalMIN (Growel Formulations Pvt. Ltd., India), containing Ca<sup>2+</sup>, Mg<sup>2+</sup>, and K<sup>+</sup>, was applied to all ponds at 10 kg ha<sup>-1</sup> every 10 days.

## 2.4 Probiotic and fermented probiotic–rice bran formulation

A commercial probiotic formulation was used in the probiotic and fermented probiotic–rice bran treatments. According to the product specification, the formulation contained *Bacillus subtilis*, *Lactobacillus acidophilus*, and *Saccharomyces cerevisiae*, with a declared viable count of 9.8×10<sup>8</sup> CFU g<sup>-1</sup>. The microbial composition and viable count were reported based on the manufacturer-provided product specification available at the time of the study. Strain-level identifiers or accession numbers were unavailable, microorganisms are reported according to the declared taxonomic information only. Therefore, the observed effects in this study are considered to be the response to the commercial probiotic mixture rather than to any specific microbial strain. Previous studies have shown that *Bacillus*-based probiotics can inhibit pathogenic *Vibrio* species and improve health conditions in penaeid shrimp farming systems (Utami *et al.* 2025).

For the SIP treatment, the probiotic was applied at a rate of 750 g ha<sup>-1</sup> every 10 days. Most of the probiotic (about 98%) was added directly to the pond water. The remaining 2% was used as a feed supplement. For feed application, 15 g of probiotic was mixed with 2.5 L of water, and the mixture was evenly sprayed onto 25 kg of feed. This coated feed was applied twice daily, four days per week, throughout the culture period.

For the SIS treatment, the formulation for a 1-ha water area consisted of 12.5 kg rice bran as the putative prebiotic substrate, 750 g Soil-Pro, 125 L water, and 5 kg calcium bicarbonate as a pH buffer. Rice bran served as the prebiotic substrate, while Soil-pro provided the microbial inoculum. The ingredients were thoroughly mixed and kept in an airtight container for 24 h at 28–32°C, followed by continuous aeration for a further 24 h. After the 48-h preparation period, 2.5 L of the formulation was used to coat 25 kg of feed, which was offered twice daily, four days per week. The remaining formulation was applied to pond water near the paddle-wheel aerators every 10 days. SIC ponds received no microbial supplementation.

## 2.5 Pond-water quality monitoring

Pond-water quality was monitored at 14-day intervals throughout the culture period. Dissolved oxygen (DO), salinity, total alkalinity, ammonia, nitrate, and morning pH were measured between 06:30 and 07:30 hr, while water temperature and afternoon pH were recorded between 16:00 and 17:00 hr. At each sampling event, meas-

urements were taken from three locations within each pond at approximately 15 – 30 cm below the water surface and averaged to obtain a single pond-level value. Diurnal pH variation ( $\Delta\text{pH}$ ) was calculated as the difference between afternoon and morning pH.

$$\Delta\text{pH} = \text{afternoon pH} - \text{morning pH}.$$

Water temperature was recorded using a WT-2 digital thermometer, while salinity was measured with an ATA-GO MASTER-S28M refractometer. pH was measured using an EZDO PH-5011 portable pH meter, which was calibrated daily with standard buffer solutions. Dissolved oxygen levels were determined using a HANNA HI3810 modified Winkler test kit. Total alkalinity, ammonia, and nitrate concentrations were measured using HANNA HI3811, HI784, and HI782 systems, respectively, according to the manufacturers' guidelines. The measured chemical parameters were expressed in  $\text{mg L}^{-1}$  where applicable.

## 2.6 Pond-soil sampling and analysis

Pond-bottom sediment samples were collected at three different stages of the culture period: before stocking (pre-culture; 30 April 2024), during culture (mid-culture; 1 August 2024, approximately 77 days after stocking), and after harvest (post-harvest; 30 October 2024, 15 days after harvest). At each sampling stage, sediment was collected from six randomly selected sites within each pond. Samples were taken from the upper 10 – 15 cm using a clean soil auger and thoroughly homogenised to obtain one representative composite sample per pond.

Composite samples were transported to the Soil Resource Development Institute (SRDI), Khulna, Bangladesh, for laboratory analysis. Prior to analysis, the samples were air-dried under shade, gently crushed, homogenized, and sieved following standard laboratory procedures.

Soil pH was measured using a glass-electrode pH meter, and electrical conductivity (EC) was determined in a 1:1 soil-to-water extract. Total nitrogen was analysed using the Kjeldahl method, organic matter by dry combustion, and available phosphorus by the modified Olsen method. Exchangeable potassium, calcium, and magnesium were determined after ammonium acetate extraction. Available sulphur and boron were extracted with calcium dihydrogen phosphate [ $\text{Ca}(\text{H}_2\text{PO}_4)_2$ ], while available zinc was extracted with diethylenetriaminepentaacetic acid (DTPA) and quantified by atomic absorption spectrophotometry (AAS). Results were expressed on a dry-weight basis. The carbon-to-nitrogen (C:N) ratio was calculated from the organic carbon and total nitrogen concentrations.

## 2.7 Enumeration of presumptive *Vibrio* spp. bacteria and TCBS colony morphotypes in pond water

Pond-water samples were collected at 14-day intervals

throughout the culture period. At each sampling event, 100 mL of near-bottom water was collected between 06:30 and 07:30 hr in sterile bottles and transported on ice to the laboratory for microbiological analysis.

Samples from each pond were processed separately using thiosulfate–citrate–bile salts–sucrose (TCBS) agar (HiMedia Laboratories, India). Tenfold serial dilutions ranging from  $10^{-3}$  to  $10^{-6}$  were prepared, and 100  $\mu\text{L}$  of an appropriate dilution was spread onto TCBS agar. Plates were incubated at  $35 \pm 1^\circ\text{C}$  for 24 hr.

Following incubation, yellow, green, and black colony morphotypes were counted separately. Yellow and green colonies were counted as presumptive *Vibrio* spp. morphotypes, whereas black colonies were recorded separately as unidentified TCBS morphotypes. Colony abundance was calculated and expressed as colony-forming units per millilitre ( $\text{CFU mL}^{-1}$ ) using the following equation:

$$\text{CFU mL}^{-1} = (\text{number of colonies} \times \text{dilution factor}) / \text{volume plated (mL)}$$

Each pond sample was analysed in duplicate, and the average count of the two plates was considered the representative value for that pond. As biochemical and molecular identification was not conducted, yellow and green colonies observed on TCBS agar were reported as presumptive *Vibrio* spp. bacteria rather than confirmed *Vibrio* species. Black colonies were treated as an unidentified morphotype and excluded from presumptive *Vibrio* spp. analysis.

## 2.8 Enumeration of selected culturable gut bacterial groups

At harvest, nine shrimp were randomly collected from each pond for enumeration of selected culturable gut bacterial groups. The gastrointestinal tracts were aseptically removed and pooled to obtain one composite gut sample per pond. The pooled gut tissue was weighed and homogenised with sterilised source water at a salinity of approximately 12 ppt. The homogenate was centrifuged at approximately 1500 rpm for 5 min, after which the supernatant was collected and serially diluted tenfold for bacterial enumeration.

Presumptive *Vibrio* spp. bacteria, presumptive *Bacillus* spp. bacteria, culturable lactobacilli, and total culturable bacteria were enumerated using TCBS Agar (M189), *Bacillus* Differentiation Agar (M1394), *Lactobacillus* MRS Agar (GM641), and Nutrient Agar (M001), respectively (HiMedia Laboratories, India). All media were prepared according to the manufacturers' instructions.

Appropriate dilutions were plated aseptically on the respective media. TCBS plates were incubated aerobically at  $37^\circ\text{C}$  for 24 hr, while plates used for the other bacterial groups were incubated aerobically at  $25 - 28^\circ\text{C}$  for 24 hr. Plates containing 30 – 300 colonies were selected for enumeration, and bacterial groups were classified accord-

ing to growth on the selective media and colony morphology (Moriarty 1998). Bacterial abundance was expressed as CFU g<sup>-1</sup> gut tissue after accounting for tissue mass, dilution factor, and inoculated volume. Counts were reported as presumptive or culturable bacterial groups according to the respective selective media and colony characteristics.

## 2.9 Growth performance, feed utilization, survival, and production

Shrimp growth was monitored at 14-day intervals throughout the 152-day culture period. At each sampling event, 100 shrimp were randomly captured from each pond using a cast net, individually weighed, and returned to their respective ponds. The measurements were used to monitor growth and adjust feed allocation during culture.

At harvest, final body weight, feed conversion ratio (FCR), survival, and total shrimp production were measured for each pond. Growth and production parameters were calculated following standard aquaculture performance indicators (Hopkins 1992). All measurements related to growth, feed utilization, survival, and production were calculated based on individual pond values.

Feed conversion ratio was calculated as:  $FCR = (\text{Total dry feed consumed (kg)} / [(\text{Final biomass (kg)} - \text{Initial Biomass (kg)})])$ .

Survival was calculated as:  $(\text{Number of shrimp harvested} / \text{Number of shrimp initially stocked}) \times 100$

Production was calculated as:  $\text{Production (kg ha}^{-1}\text{)} = \text{harvested biomass (kg)} / \text{pond area (ha)}$

## 2.10 Innate immune responses

Innate immune and antioxidant parameters were evaluated at harvest. Nine shrimp were randomly collected from each pond in the early morning and transported alive to the laboratory for analysis. After anaesthesia, haemolymph was collected from the ventral sinus using sterile syringes containing Alsever's solution, with a haemolymph-to-anticoagulant ratio of 1:1 (v/v). The haemolymph samples from the nine shrimp in each pond were pooled to form one composite sample for further analysis. The pooled samples were centrifuged at 2500 rpm to separate haemocytes.

Total haemocyte count (THC) was determined according to the method described by (Sritunyalucksana *et al.* 2005). Lysozyme activity was measured using the turbidimetric assay described by (Lee and Yang 2002). Prophenoloxidase (ProPO) activity was determined following the method of Sung *et al.* (1998) using L-3,4-dihydroxyphenylalanine (L-DOPA) as the substrate. Superoxide dismutase (SOD) activity was analysed using the spectrophotometric method described by (Beauchamp

and Fridovich 1971), with modifications for penaeid shrimp as reported by (Campa-Córdova *et al.* 2002). SOD activity was expressed as units per gram of tissue (U g<sup>-1</sup> tissue).

## 2.11 Data analysis

Statistical analyses were performed using IBM SPSS Statistics version 29 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 10.6.1. Analyses were conducted at the pond level, with three independent ponds per treatment. Measurements obtained from multiple sampling locations or individual shrimp within a pond were pooled or averaged, as appropriate, before analysis. For each water-quality variable measured at 14-day intervals, observations across sampling occasions were averaged within each pond to obtain a single overall pond-level mean; thus, repeated observations within a pond were not treated as independent replicates. Pond-water bacterial observations were similarly averaged within each pond over the culture period for treatment comparisons. Normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene tests, respectively, where applicable. Microbial counts were log<sub>10</sub>-transformed before analysis when appropriate.

Yellow and black TCBS colony morphotypes were analysed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference test. Green colony counts were analysed using Welch's ANOVA followed by Games–Howell pairwise comparisons because the homogeneity-of-variance assumption was not met.

For other endpoints, one-way ANOVA followed by Tukey's test was used when the assumptions for parametric analysis were satisfied. When homogeneity of variance was not satisfied, Welch's ANOVA followed by Games–Howell comparisons was applied. Water-quality variables and final growth and production parameters were analysed using Welch's ANOVA with Games–Howell comparisons. Ammonia concentrations were summarised descriptively because replicate ponds within each treatment had identical values.

Pond-soil variables were analysed using linear mixed-effects models. Treatment, sampling stage, and their interaction were included as fixed effects, with pond fitted as a random intercept. Models were estimated using restricted maximum likelihood, and denominator degrees of freedom were calculated using the Kenward–Roger approximation. For soil pH, an unstructured repeated covariance model was used because the random-intercept model did not converge. Significant treatment × sampling-stage interactions were examined using Bonferroni-adjusted estimated marginal means.

Pearson correlation analysis was used to examine pond-level associations among selected environmental variables, gut bacterial abundance, immune responses,

FCR, and production ( $n = 9$ ). Given the small number of pond-level observations and the multiple correlations examined, these analyses were considered exploratory. Accordingly, correlation coefficients and associated  $p$ -values were interpreted cautiously, and no causal relationships were inferred. Statistical significance was set at  $p < 0.05$ .

## 2.12 Economic analysis

Economic performance was evaluated on a hectare basis for one production cycle. Total production cost included expenditure on post-larvae, feed, microbial supplements, mineral additives, pond preparation, labour, aeration, water management, and other routine farming inputs incurred during the culture period. Gross return was calculated based on total shrimp yield and the corresponding farm-gate selling price at harvest using the following equation:

$$\text{Gross return (BDT ha}^{-1}\text{)} = \text{Production (kg ha}^{-1}\text{)} \times \text{Farm-gate price (BDT kg}^{-1}\text{)}$$

The farm-gate price was determined according to harvested shrimp size grades and the prevailing local market price at harvest which ranged from approximately BDT 960 to BDT 1,050  $\text{kg}^{-1}$  [1 USD = ~120 BDT].

Net profit was calculated as: Net profit = Gross return – Total production cost

The benefit–cost ratio (BCR) was calculated as:  $\text{BCR} = \text{Gross return} / \text{Total production cost}$

BCR was calculated separately for each replicate pond, and treatment-level values were expressed as mean  $\pm$  SD. Economic values were standardised and expressed as  $10^3$  BDT  $\text{ha}^{-1}$  production cycle $^{-1}$ .

## 3 | RESULTS

### 3.1 Changes in pond-soil physicochemical properties

Several pond-soil properties changed differently among treatments during the culture period. Significant treatment  $\times$  sampling-stage interactions were observed for soil pH, organic matter, total nitrogen, C:N ratio, and organic

carbon (Table 1; Table S3). Soil pH increased from the pre-culture stage and remained elevated at harvest across all treatments. Organic matter and organic carbon decreased under SIS but increased under SIP and SIC, with the highest post-harvest values recorded in SIC. Total nitrogen increased under SIS, whereas comparatively smaller changes were observed under SIP and SIC. The C:N ratio declined under SIS but remained relatively stable or increased in the other treatments (Figure 1; Tables S1–S3).

Electrical conductivity, phosphorus, potassium, calcium, and magnesium were mainly influenced by sampling stage, with no significant treatment  $\times$  sampling-stage interactions. Electrical conductivity and magnesium generally decreased over time, phosphorus increased during culture, and calcium remained higher at harvest than at the pre-culture stage (Tables S1–S3).

### 3.2 Water-quality parameters

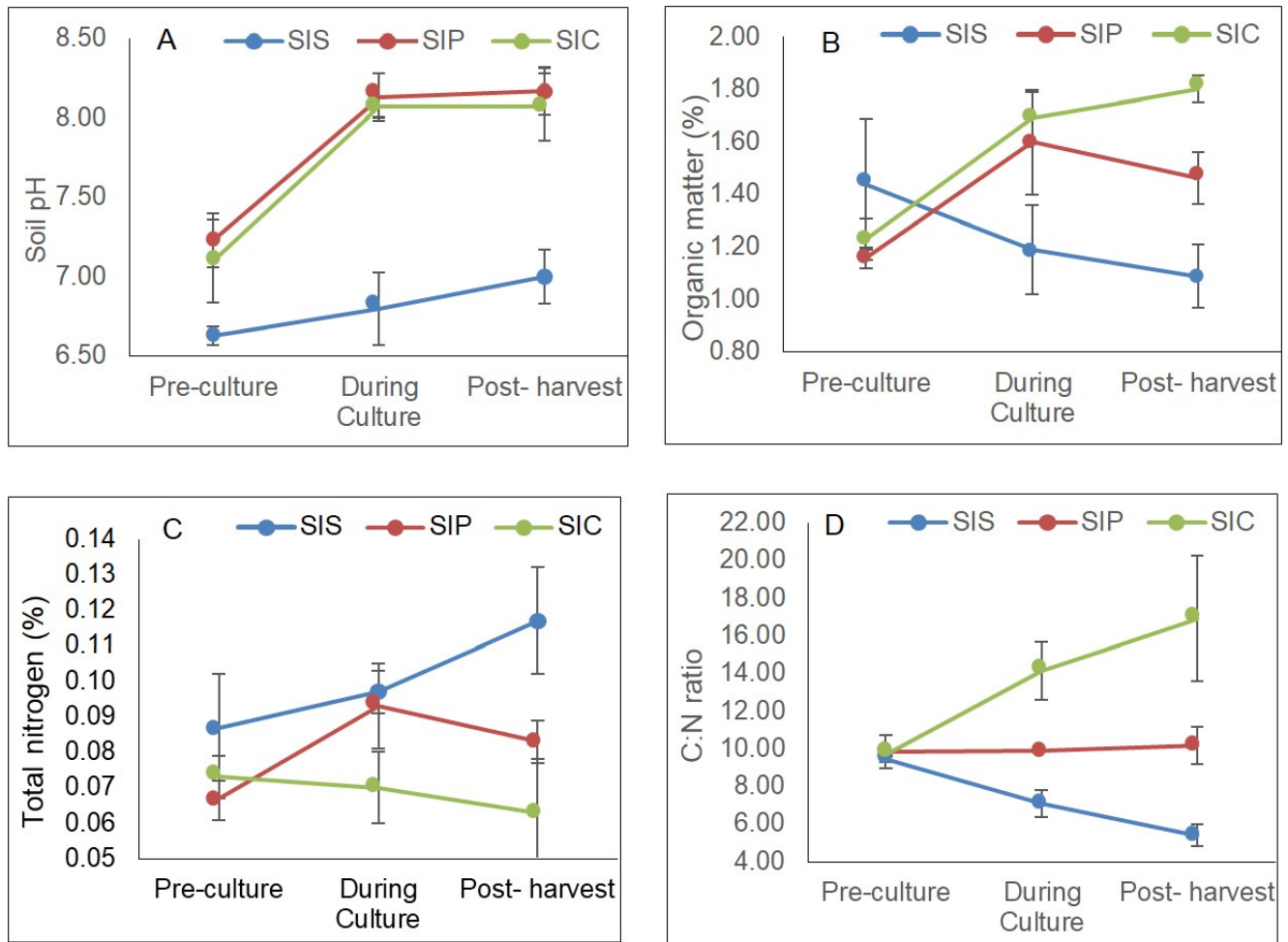
Water-quality parameters differed among microbial treatments (Table 2). Water temperature was not affected by treatment. Salinity, morning pH, evening pH, diurnal pH variation, dissolved oxygen, alkalinity, and nitrate concentration differed among treatments. SIS maintained lower evening pH and diurnal pH variation and exhibited higher dissolved oxygen compared with SIC and SIP. Alkalinity and nitrate concentrations increased progressively from SIC to SIP and SIS. Ammonia values were presented descriptively because no variation was observed among replicate ponds within treatments.

### 3.3 Presumptive *Vibrio* spp. bacteria abundance

The omnibus tests indicated significant overall treatment effects for yellow and green presumptive *Vibrio* spp. colony abundance in pond water ( $p = 0.044$  for both; Table S5). However, no individual post-hoc pairwise comparison was significant. Although SIS showed the lowest numerical abundance for both colony morphotypes, these numerical differences should not be interpreted as statistically significant pairwise treatment differences. Black colony morphotypes were analysed separately as unidentified TCBS colonies.

**TABLE 1** Effects of treatment, sampling stage, and their interaction on selected pond-soil physicochemical properties.

| Soil parameter          | Treatment effect                      | Sampling-stage effect               | Treatment $\times$ sampling-stage interaction |
|-------------------------|---------------------------------------|-------------------------------------|-----------------------------------------------|
| Soil pH                 | $F_{(2,6)} = 64.569$ ; $p < 0.001$    | $F_{(2,5)} = 204.00$ ; $p < 0.001$  | $F_{(4,5,4)} = 8.955$ ; $p = 0.014$           |
| Electrical conductivity | $F_{(2,6)} = 0.306$ ; $p = 0.747$     | $F_{(2,12)} = 1135.2$ ; $p < 0.001$ | $F_{(4,12)} = 0.753$ ; $p = 0.575$            |
| Organic matter          | $F_{(2,6)} = 7.133$ ; $p = 0.026$     | $F_{(2,12)} = 10.856$ ; $p = 0.002$ | $F_{(4,12)} = 18.420$ ; $p < 0.001$           |
| Total nitrogen          | $F_{(2, 6,0)} = 12.703$ ; $p = 0.007$ | $F_{(2,12)} = 4.531$ ; $p = 0.034$  | $F_{(4, 12)} = 5.020$ ; $p = 0.013$           |
| Phosphorus              | $F_{(2,6)} = 2.107$ ; $p = 0.203$     | $F_{(2,12)} = 11.150$ ; $p = 0.002$ | $F_{(4,12)} = 2.204$ ; $p = 0.130$            |
| C:N ratio               | $F_{(2,6)} = 27.685$ ; $p = 0.001$    | $F_{(2,12)} = 2.855$ ; $p = 0.097$  | $F_{(4, 12)} = 22.904$ ; $p < 0.001$          |

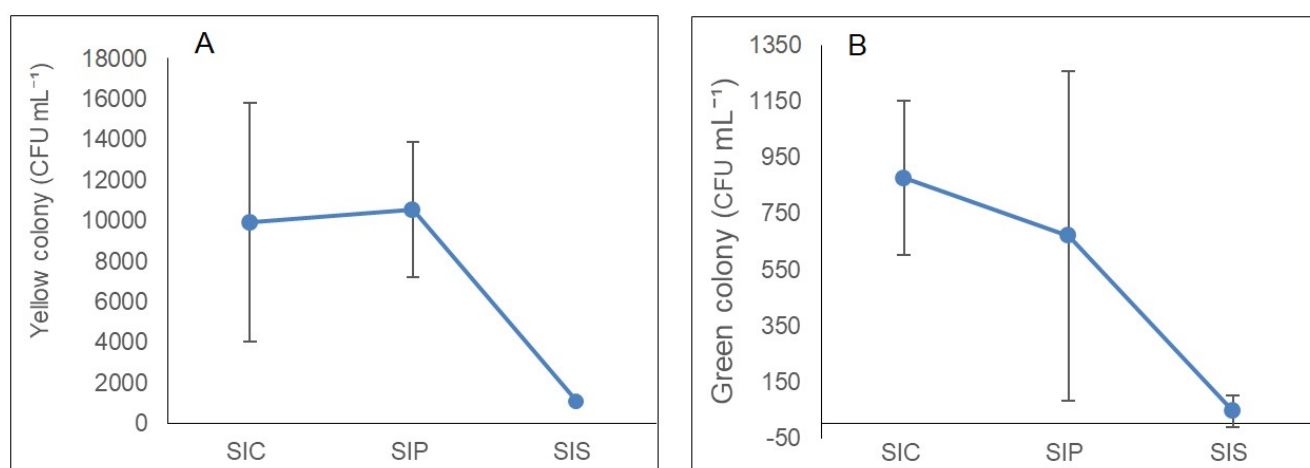


**FIGURE 1** Temporal changes in selected pond-soil physicochemical properties under control, probiotic, and fermented formulation management. Panels A–D show soil pH, organic matter (%), total nitrogen (%), and C:N ratio, respectively. Values are means  $\pm$  SD from three replicate ponds per treatment. Bonferroni-adjusted  $p < 0.05$ . SIC, semi-intensive control; SIP, semi-intensive probiotic; SIS, semi-intensive fermented probiotic-rice bran formulation.

**TABLE 2** Water-quality characteristics of semi-intensive *Penaeus monodon* ponds under different microbial treatments.

| Parameter                                            | SIC                           | SIP                           | SIS                           |
|------------------------------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Temperature ( $^{\circ}\text{C}$ )                   | 31.20 $\pm$ 0.10 <sup>a</sup> | 30.97 $\pm$ 0.76 <sup>a</sup> | 31.40 $\pm$ 0.20 <sup>a</sup> |
| Salinity (ppt)                                       | 9.48 $\pm$ 0.30 <sup>ab</sup> | 10.22 $\pm$ 0.22 <sup>a</sup> | 9.53 $\pm$ 0.19 <sup>b</sup>  |
| pH, morning                                          | 8.08 $\pm$ 0.19 <sup>a</sup>  | 7.88 $\pm$ 0.16 <sup>a</sup>  | 7.57 $\pm$ 0.11 <sup>a</sup>  |
| pH, evening                                          | 8.89 $\pm$ 0.12 <sup>a</sup>  | 8.58 $\pm$ 0.08 <sup>b</sup>  | 7.88 $\pm$ 0.12 <sup>c</sup>  |
| $\Delta\text{pH}$                                    | 0.81 $\pm$ 0.08 <sup>a</sup>  | 0.70 $\pm$ 0.10 <sup>a</sup>  | 0.31 $\pm$ 0.03 <sup>b</sup>  |
| DO ( $\text{mg L}^{-1}$ )                            | 5.61 $\pm$ 0.09 <sup>b</sup>  | 5.74 $\pm$ 0.07 <sup>b</sup>  | 6.50 $\pm$ 0.10 <sup>a</sup>  |
| Ammonia ( $\text{mg L}^{-1}$ )                       | 0.05 $\pm$ 0.00               | 0.01 $\pm$ 0.00               | 0.00 $\pm$ 0.00               |
| Alkalinity ( $\text{mg L}^{-1}$ as $\text{CaCO}_3$ ) | 101 $\pm$ 2 <sup>c</sup>      | 110 $\pm$ 2 <sup>b</sup>      | 145 $\pm$ 2 <sup>a</sup>      |
| Nitrate ( $\text{mg L}^{-1}$ )                       | 1.60 $\pm$ 0.02 <sup>c</sup>  | 2.10 $\pm$ 0.10 <sup>b</sup>  | 2.70 $\pm$ 0.10 <sup>a</sup>  |

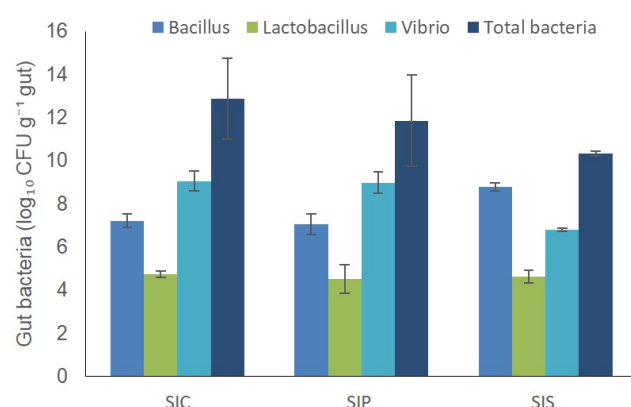
Values are presented as pond-level means  $\pm$  SD calculated from fortnightly measurements, with three independent ponds per treatment. Treatment effects were evaluated using Welch's one-way ANOVA followed by Games–Howell pairwise comparisons. Within a row, means without a common superscript letter differ significantly at  $p < 0.05$ . Ammonia was reported descriptively because zero within-treatment variance prevented valid inferential analysis. SIC, semi-intensive control; SIP, semi-intensive probiotic; SIS, semi-intensive fermented probiotic-rice bran formulation; DO, dissolved oxygen;  $\Delta\text{pH}$ , difference between evening and morning pH.



**FIGURE 2** Presumptive *Vibrio* spp. bacteria in pond water under different microbial treatments. Panels A and B show yellow and green presumptive *Vibrio* spp. colony morphotypes, respectively. Values are means  $\pm$  SD ( $n = 3$  ponds per treatment). The omnibus tests indicated significant overall treatment effects for yellow and green colony morphotypes ( $p = 0.044$  for both); however, no individual post-hoc pairwise comparison was significant at  $p < 0.05$ . SIC, semi-intensive control; SIP, semi-intensive probiotic; SIS, semi-intensive fermented probiotic-rice bran formulation.

### 3.4 Gut bacterial populations

Gut bacterial populations differed among treatments (Figure 3; Table S6). Presumptive *Bacillus*-like bacterial abundance was significantly higher in SIS compared with SIC and SIP. In contrast, presumptive *Vibrio* spp. bacterial abundance was significantly lower in SIS than in SIC and SIP. No significant differences were detected among treatments for *Lactobacillus* or total culturable bacterial abundance.



**FIGURE 3** Selected culturable gut bacterial groups of *Penaeus monodon* under different microbial treatments. Values are means  $\pm$  SD ( $n = 3$  ponds per treatment). Treatment effects were significant for presumptive *Bacillus* spp. and *Vibrio* spp. bacteria ( $p < 0.05$ ), whereas *Lactobacillus* and total bacterial abundance did not differ significantly among treatments.

### 3.5 Innate immune responses

Innate immune and antioxidant parameters were signifi-

cantly influenced by microbial treatments (Figure 4; Table S7). SIS exhibited higher total haemocyte count, lysozyme activity, prophenoloxidase activity, and superoxide dismutase activity compared with SIP and SIC. Significant treatment effects were observed for all measured immune and antioxidant parameters.

### 3.6 Growth performance and production

Growth performance, feed utilization, survival, and production parameters differed among treatments (Table 3). SIS resulted in the highest final body weight and production, together with the lowest feed conversion ratio. Shrimp survival was higher in microbial treatments compared with SIC.

### 3.7 Economic performance

Economic performance differed among treatments (Table 4). SIS had higher production costs due to additional formulation inputs but generated the highest gross return, net profit, and benefit–cost ratio. SIP showed intermediate economic performance, whereas SIC resulted in the lowest economic return.

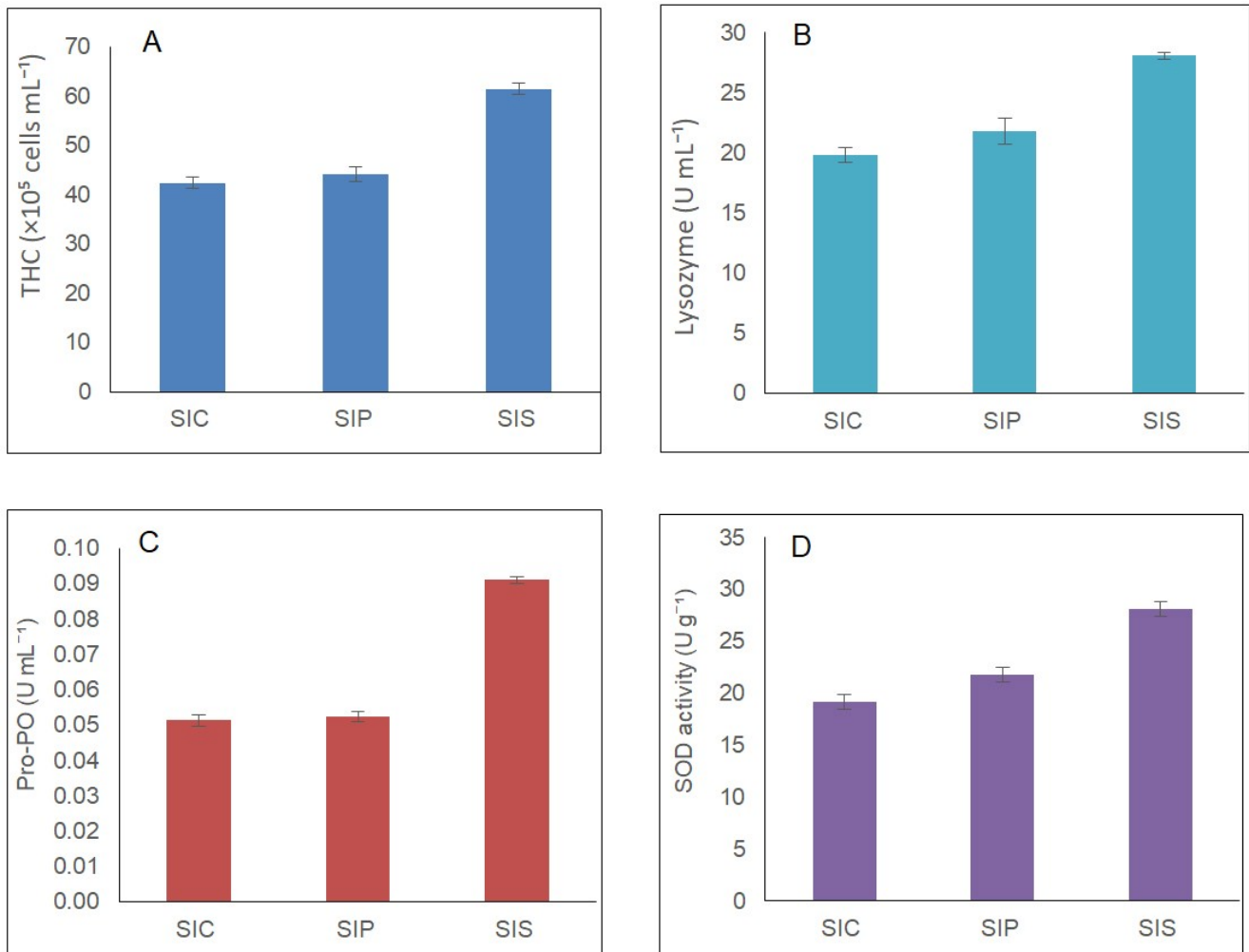
### 3.8 Exploratory associations among environmental, microbial, immune, and production responses

Pearson correlation analysis was performed to evaluate associations among environmental, microbial, immune, and production variables (Table S8). Dissolved oxygen (DO) showed positive correlations with total haemocyte count (THC), lysozyme activity, prophenoloxidase (ProPO) activity, and production performance ( $r = 0.972 - 0.979$ ), and a negative correlation with feed conversion ratio (FCR) ( $r = -0.894$ ). These correlations should be regarded as exploratory associations because they were based on

only nine pond-level observations and multiple correlations were examined; therefore, they should not be interpreted as evidence of causal relationships.

Changes in soil organic matter ( $\Delta$  soil OM) showed negative correlations with immune parameters and production performance ( $r = -0.915$  to  $-0.950$ ) and a positive correlation with FCR ( $r = 0.942$ ). Gut presumptive *Bacillus*

spp. bacterial abundance showed positive correlations with immune indicators and production performance ( $r = 0.854 - 0.928$ ) and a negative correlation with FCR ( $r = -0.751$ ). In contrast, gut presumptive *Vibrio* spp. bacterial abundance showed negative correlations with immune responses and production performance ( $r = -0.910$  to  $-0.960$ ) and a positive correlation with FCR ( $r = 0.823$ ).



**FIGURE 4** Innate immune responses of *Penaeus monodon* under different microbial treatments. Panels a–d represent total haemocyte count (THC), lysozyme activity, prophenoloxidase (ProPO) activity, and superoxide dismutase (SOD) activity, respectively. Values are presented as means  $\pm$  SD ( $n = 3$  ponds per treatment,  $p < 0.05$ ). SIC, semi-intensive control; SIP, semi-intensive probiotic treatment; SIS, semi-intensive fermented probiotic–rice bran formulation treatment.

**TABLE 3** Growth performance, survival, and production of *Penaeus monodon* under different microbial treatments.

| Parameter                          | SIC                              | SIP                               | SIS                              |
|------------------------------------|----------------------------------|-----------------------------------|----------------------------------|
| Final body weight (g)              | 54.37 $\pm$ 0.99 <sup>b</sup>    | 56.48 $\pm$ 1.08 <sup>b</sup>     | 62.50 $\pm$ 0.85 <sup>a</sup>    |
| Feed conversion ratio (FCR)        | 1.793 $\pm$ 0.021 <sup>a</sup>   | 1.700 $\pm$ 0.030 <sup>b</sup>    | 1.557 $\pm$ 0.040 <sup>c</sup>   |
| Survival (%)                       | 65.33 $\pm$ 0.58 <sup>b</sup>    | 67.00 $\pm$ 2.00 <sup>ab</sup>    | 70.67 $\pm$ 0.58 <sup>a</sup>    |
| Production ( $\text{kg ha}^{-1}$ ) | 4913.30 $\pm$ 32.51 <sup>b</sup> | 5234.67 $\pm$ 116.60 <sup>b</sup> | 6111.67 $\pm$ 13.20 <sup>a</sup> |

Note: Values are means  $\pm$  SD ( $n = 3$  ponds per treatment). Different superscript letters within a row indicate significant differences among treatments based on Games–Howell comparisons ( $p < 0.05$ ). Treatment effects were evaluated using Welch's ANOVA. SIC, semi-intensive control; SIP, semi-intensive probiotic; SIS, semi-intensive fermented probiotic–rice bran formulation.

**TABLE 4** Economic performance of *Penaeus monodon* under different microbial treatments.

| Economic parameter                                                       | SIC                          | SIP                          | SIS                          |
|--------------------------------------------------------------------------|------------------------------|------------------------------|------------------------------|
| Total production cost ( $10^3$ Tk ha <sup>-1</sup> cycle <sup>-1</sup> ) | 3075.00                      | 3302.00                      | 3440.00                      |
| Production (kg ha <sup>-1</sup> )                                        | 4913.00                      | 5235.00                      | 6112.00                      |
| Gross return ( $10^3$ Tk ha <sup>-1</sup> cycle <sup>-1</sup> )          | 4814.74                      | 5496.75                      | 6416.55                      |
| Net profit ( $10^3$ Tk ha <sup>-1</sup> cycle <sup>-1</sup> )            | 1739.74                      | 2194.75                      | 2976.55                      |
| BCR (mean $\pm$ SD)                                                      | 1.57 $\pm$ 0.02 <sup>c</sup> | 1.66 $\pm$ 0.00 <sup>b</sup> | 1.87 $\pm$ 0.03 <sup>a</sup> |

Note: BCR values are means  $\pm$  SD ( $n = 3$  ponds per treatment). Different superscript letters indicate significant differences among treatments ( $p < 0.05$ ). SIC, semi-intensive control; SIP, semi-intensive probiotic; SIS, semi-intensive fermented probiotic-rice bran formulation.

## 4 | DISCUSSION

### 4.1 Effects of microbial supplementation on pond-soil characteristics

The main sediment responses were observed for organic matter, organic carbon, total nitrogen, and the C:N ratio. Under SIS, organic matter and organic carbon declined from pre-culture to post-harvest, accompanied by a reduction in the C:N ratio, whereas organic matter increased under SIC. These findings indicate that sediment nutrient dynamics varied among the treatments. Probiotic microorganisms may contribute to organic matter decomposition and nutrient transformation processes in aquaculture systems (Verschuere *et al.* 2000). Previous studies have also reported that synbiotic formulations can improve pond environmental conditions (Shinde *et al.* 2023).

The decrease in the C:N ratio, together with the increase in total nitrogen observed under SIS, suggests changes in carbon and nitrogen cycling during the culture period. However, microbial biomass, mineralisation rates, and specific nutrient transformation pathways were not measured; therefore, the mechanisms responsible for these sediment changes cannot be attributed to specific microbial processes. Further investigations integrating microbial community analysis and nutrient-flow measurements would help clarify these interactions.

### 4.2 Effects of microbial supplementation on water-quality regulation

The greatest differences in water quality were observed in SIS ponds, which maintained higher dissolved oxygen and alkalinity, lower evening pH, and reduced diurnal pH variation compared with SIC and SIP. Microbial supplementation can influence pond water quality through modification of organic matter degradation and nutrient cycling processes (Verschuere *et al.* 2000; Goh *et al.* 2022), and improved regulation of nitrogenous compounds following microbial application has been reported in shrimp culture systems (Shinde *et al.* 2023; Utami *et al.* 2025).

SIS ponds showed higher nitrate and lower ammonia concentrations, indicating differences in nitrogen dynam-

ics among treatments. However, ammonia was analysed descriptively because of zero within-treatment variance, and the present measurements do not distinguish among nitrification, assimilation, or other nitrogen-transformation pathways. Higher alkalinity and lower pH fluctuation in SIS may have resulted from the combined effects of the complete formulation, including the buffering role of calcium bicarbonate, rather than microbial activity alone.

### 4.3 Growth performance and production responses

Growth performance and feed utilization improved in the SIS treatment, which agrees with previous studies reporting better growth, feed conversion ratio, and survival in penaeid shrimp after fermented microbial supplementation (Kewcharoen and Srisapoom 2022; Chin *et al.* 2024; Widyaning *et al.* 2024). The higher production observed under SIS was associated with improvements in pond conditions, gut bacterial composition, and shrimp physiological responses.

The probiotic treatment alone showed some positive effects; however, these improvements were less consistent than those observed in SIS. The SIS formulation contained several components, including probiotic microorganisms, rice bran, calcium bicarbonate, fermentation, and a modified application method. Therefore, the individual contribution of each component to improved growth performance could not be determined under the current experimental design.

### 4.4 Modulation of presumptive *Vibrio* spp. bacterial groups

Yellow and green presumptive *Vibrio* spp. colonies in pond water showed the lowest numerical abundance in the SIS treatment. Although the omnibus tests indicated significant overall treatment effects for yellow and green presumptive *Vibrio* spp. colonies, no individual post-hoc pairwise comparison was significant. Thus, the omnibus results indicate overall variation among treatments, but the pairwise analyses do not identify statistically significant differences between specific treatments. Consequently, the lower numerical abundance observed in SIS

should not be interpreted as evidence of significant suppression of presumptive *Vibrio* spp. Bacteria relative to SIC or SIP.

Since bacterial identification was based only on colony characteristics on TCBS agar, the observed counts represent presumptive culture-recoverable groups rather than confirmed *Vibrio* species. Beneficial microorganisms may affect opportunistic *Vibrio* populations through competition for nutrients and attachment sites or through the production of antimicrobial compounds (Verschuere *et al.* 2000; Goh *et al.* 2022). Previous studies have also reported that *Bacillus*-based supplementation may reduce *Vibrio* pressure in shrimp culture systems (Vaseeharan and Ramasamy 2003; Utami *et al.* 2025;). However, molecular identification and targeted microbial analyses are needed to confirm these mechanisms.

#### 4.5 Gut microbial modulation by the SIS formulation

The effects of SIS on gut bacterial groups were more pronounced than those observed in pond water. SIS increased presumptive *Bacillus* spp. bacterial abundance and reduced presumptive *Vibrio* spp. bacterial abundance in the shrimp gut, while culturable lactobacilli and total culturable bacterial counts remained unchanged. These findings suggest that SIS affected the composition of specific culturable bacterial groups rather than causing a general increase in total bacterial abundance.

Previous studies have shown that fermented probiotic–rice bran formulations can modify intestinal bacterial communities in shrimp. Similar changes in gut bacterial profiles have been reported following the application of combined microbial supplements in *P. monodon* (Hasyimi *et al.* 2020; Goh *et al.* 2022; Khanjani *et al.* 2024). Similarly, combined microbial supplementation has been associated with improved intestinal bacterial profiles in *P. monodon* (Chin *et al.* 2024). The increased abundance of presumptive *Bacillus* spp. bacteria and reduced presumptive *Vibrio* spp. bacteria observed under SIS indicate a shift in culturable gut bacterial composition.

However, culture-based enumeration represents only a fraction of the intestinal microbial community. Molecular identification and high-throughput sequencing approaches would provide a more comprehensive understanding of microbial changes associated with SIS application.

#### 4.6 Enhancement of innate immune responses

SIS-treated shrimp showed higher total haemocyte count (THC), lysozyme activity, prophenoloxidase (ProPO) activity, and superoxide dismutase (SOD) activity, indicating enhanced measured innate immune and antioxidant responses. The ProPO system is a key component of crustacean innate immunity (Amparyup *et al.* 2013), whereas SOD plays an important role in regulating oxidative stress responses in *P. monodon* (Duan *et al.* 2015). Similar im-

provements in immune-related parameters following fermented probiotic–rice bran supplementation have also been reported in penaeid shrimp (Sritunyalucksana *et al.* 2005; Huynh *et al.* 2018; Kewcharoen and Srisapoom 2022).

Although the immune and antioxidant responses observed in SIS-treated shrimp indicate changes in physiological status, they cannot be considered direct evidence of enhanced disease resistance. Pathogen-challenge experiments are required to determine whether these responses provide improved protection against specific pathogens.

#### 4.7 Ecological implications of the SIS formulation

The present study evaluated the effects of a fermented probiotic–rice bran formulation on several components of the shrimp culture system, including sediment quality, water conditions, gut bacterial groups, immune responses, feed utilization, production performance, and economic outcomes throughout a complete semi-intensive production cycle. This integrated evaluation provides a broader understanding of microbial-based management strategies under practical farm conditions compared with studies examining individual response parameters. Improvements in sediment quality and water stability were observed together with changes in gut bacterial groups, immune responses, feed efficiency, and production performance. These results indicate that SIS influenced several interconnected processes within the shrimp culture system. However, given the limited number of ponds and the multi-component nature of SIS, these responses should be interpreted as associations rather than evidence of specific causal mechanisms. Further farm-scale studies incorporating component-specific treatments are required to clarify the underlying processes.

#### 4.8 Economic feasibility of the SIS formulation

The biological responses observed under SIS were accompanied by improved economic performance. Although SIS required higher production costs due to additional formulation inputs, the increased shrimp production resulted in higher gross return, net profit, and benefit–cost ratio. Economic evaluation is particularly important for determining whether microbial interventions provide practical value under commercial farming conditions.

Economic assessments remain limited in studies evaluating probiotic and prebiotic applications in shrimp farming (Noman *et al.* 2024). A strength of the present analysis is that profitability was assessed over a complete production cycle under semi-intensive pond conditions rather than short-term laboratory conditions. However, these estimates represent a single production cycle and may vary with feed price, input costs, shrimp size grade, market conditions, and farm-management practices.

#### 4.9 Limitations and future perspectives

This study was conducted at a single farm over one production cycle, with only three independent ponds per treatment. The limited number of replicate ponds restricts statistical power and may limit the generalizability of the findings beyond the specific farm and production conditions evaluated. Therefore, the results should be interpreted with appropriate caution, and multi-farm, multi-season studies with larger numbers of independent replicate ponds are required to validate whether the observed responses remain consistent across different farming conditions. The bacterial analyses were based on culture-dependent methods, which limited species-level identification and characterization of the wider microbial community. Future studies incorporating molecular identification and high-throughput sequencing would provide deeper insights into microbial responses, particularly those involving presumptive *Vibrio* spp. bacteria.

SIS represented a multi-component intervention involving commercial probiotics, rice bran, calcium bicarbonate, fermentation, and a modified application strategy. Therefore, the contribution of individual components and specific probiotic–prebiotic interactions could not be separated under the present experimental design. Component-specific experiments are needed to clarify their individual and combined effects.

The exploratory correlations were based on only nine pond-level observations and involved multiple comparisons, which increases uncertainty and the possibility of false-positive associations. Therefore, these correlations should be interpreted cautiously as preliminary associations rather than evidence of causal relationships. In addition, the economic evaluation represented a single production cycle. Despite these limitations, the integrated responses observed across environmental conditions, microbial profiles, immune responses, production performance, and economic outcomes support further farm-scale evaluation of the fermented formulation in semi-intensive *P. monodon* culture.

## 5 | CONCLUSIONS

The fermented formulation was associated with improvements in pond conditions, selected gut bacterial groups, shrimp physiological responses, feed efficiency, and economic performance in semi-intensive *Penaeus monodon* culture. The greater responses observed under SIS compared with probiotic supplementation alone suggest that the combined formulation may provide additional benefits under farm conditions. This study highlights the importance of evaluating microbial formulations using an integrated approach that considers environmental, microbial, physiological, and economic responses rather than production outcomes alone. As SIS involved multiple components, future studies incorporat-

ing component-specific controls, molecular analyses, and multi-farm trials are needed to clarify the mechanisms involved and validate its effectiveness across different farming conditions.

## ACKNOWLEDGEMENTS

The authors sincerely thank the participating shrimp farmers for their valuable cooperation and for granting access to their ponds and field facilities throughout the study period. The authors also gratefully acknowledge the support of the Sustainable Coastal and Marine Fisheries Project, Department of Fisheries, Bangladesh, for providing partial funding for this research. This research was supported by the Sustainable Coastal and Marine Fisheries Project, Department of Fisheries, Bangladesh [Grant No. L4W1-14]. The funding agency had no involvement in the study design, data collection, data analysis, interpretation of results, decision to publish, or preparation of the manuscript.

## ETHICAL APPROVAL

The study was conducted under institutional ethical clearance issued by the Fisheries and Marine Resource Technology (FMRT) Discipline, Khulna University, Bangladesh (Ref. No.: FMRT/KU/ETH/001/2025; Date: 12 November 2025). The research involved cultured *P. monodon* maintained under semi-intensive farm conditions. For assessment of innate immune and antioxidant responses at harvest, nine shrimp were randomly collected from each pond and transported alive to the laboratory. Before haemolymph collection, the shrimp were anaesthetized to minimize handling stress and discomfort. Haemolymph was subsequently collected from the ventral sinus using sterile syringes containing Alsever's solution. The haemolymph samples from the nine shrimp collected from each pond were pooled to form one composite sample for subsequent analyses of total haemocyte count, lysozyme activity, prophenoloxidase activity, and superoxide dismutase activity. The animals used for haemolymph collection were handled only to the extent necessary to obtain the biological samples required for the study, and appropriate measures were taken to minimize stress and unnecessary distress. The study did not involve human participants, human biological materials, or personally identifiable human data, and no endangered, threatened, protected, or wild animal species were involved.

## CONFLICT OF INTEREST

The author declares no conflict of interest.

## AUTHORS' CONTRIBUTION

Saroj Kumar Mistry contributed to the conceptualization, investigation, methodology, formal analysis, data curation, and the preparation of the original draft. Syed Ha-

fizur Rahman was responsible for funding acquisition, project administration, supervision, visualization and the review and editing of the manuscript. Md. Golam Sarower contributed to the methodology, supervision, and review and editing of the manuscript.

## AI-USE STATEMENT

The authors declare that AI tools were used only for language editing, grammar refinement, and improving manuscript clarity. AI tools were not used for data generation, analysis, interpretation of results, or study-related decision-making. The authors remain fully responsible for the accuracy, originality, and integrity of the manuscript.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on a reasonable request from the corresponding author.

## REFERENCES

- Abdullah AN, Myers B, Stacey N, Zander KK, Garnett ST (2017) [The impact of the expansion of shrimp aquaculture on livelihoods in coastal Bangladesh](#). *Environment, Development and Sustainability* 19(5): 2093–2114.
- Ahmed N, Diana JS (2015) [Threatening “white gold”: Impacts of climate change on shrimp farming in coastal Bangladesh](#). *Ocean & Coastal Management* 114: 42–52.
- Alam SMN (2023) [Advancing quality and health management practices in extensive shrimp \(\*Penaeus monodon\*\) farming in Bangladesh](#). *Aquaculture International* 31(1): 1–13.
- Amparyup P, Charoensapsri W, Tassanakajon A (2013) [Prophenoloxidase system and its role in shrimp immune responses against major pathogens](#). *Fish & Shellfish Immunology* 34(4): 990–1001.
- Azad AK, Jensen KR, Lin CK (2009) [Coastal aquaculture development in Bangladesh: unsustainable and sustainable experiences](#). *Environmental Management* 44(4): 800–809.
- Beauchamp C, Fridovich I (1971) [Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels](#). *Analytical Biochemistry* 44(1): 276–287.
- Boyd CE, Tucker CS (1998) [Pond aquaculture water quality management](#). Springer US.
- Cabello FC (2006) [Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment](#). *Environmental Microbiology* 8(7): 1137–1144.
- Campa-Córdova AI, Hernández-Saavedra NY, De Philippis R, Ascencio F (2002) [Generation of superoxide anion and SOD activity in haemocytes and muscle of American white shrimp \(\*Litopenaeus vannamei\*\) as a response to  \$\beta\$ -glucan and sulphated polysaccharide](#). *Fish & Shellfish Immunology* 12(4): 353–366.
- Chen J, Lei S (1990) [Toxicity of ammonia and nitrite to \*Penaeus monodon\* juveniles](#). *Journal of the World Aquaculture Society* 21(4): 300–306.
- Chin YK, Haifa-Haryani WO, Nazarudin MF, Ahmad MI, Azzam-Sayuti M, ... Ina-Salwany MY (2024) [The synergistic \*Lactobacillus plantarum\* L<sub>20</sub> and \*Sargassum polycystum\* -added diet for improvement of Black tiger shrimp, \*Penaeus monodon\*'s growth, immune responses, bacterial profiles, and resistance against \*Vibrio parahaemolyticus\* associated acute hepatopancreatic necrosis disease \(AHPND\) infection](#). *Aquaculture Reports* 34: 101903.
- Cornejo-Granados F, Lopez-Zavala AA, Gallardo-Becerra L, Mendoza-Vargas A, Sánchez F, ... Ochoa-Leyva A (2017) [Microbiome of Pacific whiteleg shrimp reveals differential bacterial community composition between wild, aquacultured and AHPND/EMS outbreak conditions](#). *Scientific Reports* 7(1): 11783.
- Duan Y, Zhang J, Dong H, Wang Y, Liu Q, Li H (2015) [Oxidative stress response of the black tiger shrimp \*Penaeus monodon\* to \*Vibrio parahaemolyticus\* challenge](#). *Fish & Shellfish Immunology* 46(2): 354–365.
- Goh JXH, Tan LT, Law JW, Ser H, Khaw K, ... Goh B (2022) [Harnessing the potentialities of probiotics, prebiotics, synbiotics, paraprobiotics, and postbiotics for shrimp farming](#). *Reviews in Aquaculture* 14(3): 1478–1557.
- Han S, Wang B, Wang M, Liu Q, Zhao W, Wang L (2017) [Effects of ammonia and nitrite accumulation on the survival and growth performance of white shrimp \*Litopenaeus vannamei\*](#). *Invertebrate Survival Journal* 14(1): 221–232.
- Hasyimi W, Widanarni W, Yuhana M (2020) [Growth performance and intestinal microbiota diversity in Pacific white shrimp \*Litopenaeus vannamei\* fed with a probiotic bacterium, honey prebiotic, and synbiotic](#). *Current Microbiology* 77(10): 2982–2990.
- Hopkins KD (1992) [Reporting fish growth: a review of the basics](#). *Journal of the World Aquaculture Society* 23(3): 173–179.
- Huynh T-G, Cheng A-C, Chi C-C, Chiu K-H, Liu C-H (2018) [A synbiotic improves the immunity of white shrimp, \*Litopenaeus vannamei\*: metabolomic analysis reveal compelling evidence](#). *Fish & Shellfish Immunology* 79: 284–293.
- Kewcharoen W, Srisapoom P (2022) [Potential synbiotic effects of a \*Bacillus\* mixture and chitosan on growth, immune responses and VP\(AHPND\) resistance in Pacific white shrimp \(\*Litopenaeus vannamei\*, Boone, 1931\)](#). *Fish & Shellfish Immunology* 127: 715–729.
- Khanjani MH, Mozanzadeh MT, Gisbert E, Hoseinifar SH (2024) [Probiotics, prebiotics, and synbiotics in shrimp aquaculture: their effects on growth performance, immune responses, and gut microbiome](#).

- Aquaculture Reports 38: 102362.
- Lee YC and Yang D (2002) [Determination of lysozyme activities in a microplate format](#). Analytical Biochemistry 310(2): 223–224.
- Lin L, Li J, Liu J, Zhuo H, Zhang Y, ... Zhao X (2024) [Single and combined effects of ammonia and nitrite on \*Litopenaeus vannamei\*: histological, physiological and molecular responses](#). Aquaculture Reports 35: 102014.
- Mathan Muthu CM, Vickram AS, Bhavani Sowndharya B, Saravanan A, Kamalesh R, Dinakarkumar Y (2024) [A comprehensive review on the utilization of probiotics in aquaculture towards sustainable shrimp farming](#). Fish & Shellfish Immunology 147: 109459.
- Moriarty DJW (1998) [Control of luminous \*Vibrio\* species in penaeid aquaculture ponds](#). Aquaculture 164(1–4): 351–358.
- Noman M, Kazmi SSUH, Saqib HSA, Fiaz U, Pastorino P, ... Yaseen ZM (2024) [Harnessing probiotics and prebiotics as eco-friendly solution for cleaner shrimp aquaculture production: a state of the art scientific consensus](#). Science of The Total Environment 915: 169921.
- Rahman R, Ando K, Takeda S (2014) [Development of shrimp-based cropping systems in the coastal area of Bangladesh: a village-level study in Satkhira district](#). Journal of Land Use Science 9(2): 195–210.
- Rajeev R, Adithya KK, Kiran GS, Selvin J (2021) [Healthy microbiome: a key to successful and sustainable shrimp aquaculture](#). Reviews in Aquaculture 13(1): 238–258.
- Rohani MF, Islam SM, Hossain MK, Ferdous Z, Siddik MAb, ... Shahjahan M (2022) [Probiotics, prebiotics and synbiotics improved the functionality of aquafeed: Upgrading growth, reproduction, immunity and disease resistance in fish](#). Fish & Shellfish Immunology 120: 569–589.
- Rungrassamee W, Klanchui A, Chaiyapechara S, Maibunkaew S, Tangphatsornruang S, ... Karoonuthaisiri N (2013) [Bacterial population in intestines of the black tiger shrimp \(\*Penaeus monodon\*\) under different growth stages](#). PLoS ONE 8(4): e60802.
- Rungrassamee W, Klanchui A, Maibunkaew S, Chaiyapechara S, Jiravanichpaisal P, Karoonuthaisiri N (2014) [Characterization of intestinal bacteria in wild and domesticated adult black tiger shrimp \(\*Penaeus monodon\*\)](#). PLoS ONE 9(3): e91853.
- Shinde AH, Sonpal V, Maiti P, Halder S (2023) [Evaluation of a synbiotic formulation for water remediation in a shrimp pond](#). Environmental Science and Pollution Research 30(24): 65990–66001.
- Sritunyalucksana K, Gangnonngiw W, Archakunakorn S, Fegan D, Flegel T (2005) [Bacterial clearance rate and a new differential hemocyte staining method to assess immunostimulant activity in shrimp](#). Diseases of Aquatic Organisms 63: 89–94.
- Sung H-H, Chang H-J, Her C-H, Chang J-C, Song Y-L (1998) [Phenoloxidase activity of hemocytes derived from \*Penaeus monodon\* and \*Macrobrachium rosenbergii\*](#). Journal of Invertebrate Pathology 71(1): 26–33.
- Swanson KS, Gibson GR, Hutkins R, Reimer RA, Reid G, ... Sanders ME (2020) [The International Scientific Association for Probiotics and Prebiotics \(ISAPP\) consensus statement on the definition and scope of synbiotics](#). Nature Reviews Gastroenterology & Hepatology 17(11): 687–701.
- Utami DAS, Ilham I, Insani L, Kusmiatun A, Wahyu W, ... Szuzter BW (2025) [Commercial probiotics improve growth, feed efficiency, nitrogen removal, hemocyte count and suppression of population in Pacific white shrimp culture](#). ILMU KELAUTAN: Indonesian Journal of Marine Sciences 30(3): 395–410.
- Vaseeharan B, Ramasamy P (2003) [Control of pathogenic \*Vibrio\* spp. by \*Bacillus subtilis\* BT23, a possible probiotic treatment for black tiger shrimp \*Penaeus monodon\*](#). Letters in Applied Microbiology 36(2): 83–87.
- Verschuere L, Rombaut G, Sorgeloos P, Verstraete W (2000) [Probiotic bacteria as biological control Agents in aquaculture](#). Microbiology and Molecular Biology Reviews 64(4): 655–671.
- Wahab MA, Bergheim A, Braaten B (2003) [Water quality and partial mass budget in extensive shrimp ponds in Bangladesh](#). Aquaculture 218(1–4): 413–423.
- Widanarni, Widagdo P, Wahjuningrum D (2013) [Oral application of probiotic, prebiotic, and synbiotic in Pacific white shrimp \(\*Litopenaeus vannamei\*\) challenged with \*Vibrio harveyi\*](#). Jurnal Akuakultur Indonesia 11(1): 54.
- Widyany DA, Prayitno SB, Desrina (2024) [Effect of probiotics, prebiotics, synbiotics on survival, growth, and immune response of white shrimp \(\*Litopenaeus vannamei\*\) infected with \*Vibrio parahaemolyticus\*](#). Asia Pacific Journal of Molecular Biology and Biotechnology 32(3): 98–112.



SK Mistry  <http://orcid.org/0009-0008-9107-0720>

SH Rahman  <http://orcid.org/0000-0003-0112-9124>

MG Sarower  <http://orcid.org/0000-0001-6488-9839>