***Original Research Article***

**Synergistic Effects of Probiotics and Oligosaccharide Prebiotics on Water Quality and Growth Performance of *Litopenaeus vannamei***

The title of the research express the content of the research

**ABSTRACT**

The increasing intensification of shrimp aquaculture, particularly of *Litopenaeus vannamei*, necessitates sustainable strategies to enhance growth performance while maintaining optimal water quality. This study investigates the synergistic effects of dietary supplementation with probiotics and prebiotics—specifically Mannan-oligosaccharides (MOS), Fructo-oligosaccharides (FOS), and Xylo-oligosaccharides (XOS)—administered individually and in various combinations on the growth, survival, feed utilization, and water quality parameters in *L. vannamei* cultured under controlled biofloc conditions.

Twelve experimental diets were formulated: a control diet (T1), basal diets supplemented with either probiotics or individual prebiotics (T2–T5), and various synbiotic combinations of probiotics with one or more prebiotics (T6–T12). The feeding trial was conducted for 80 days using triplicate groups per treatment, with regular monitoring of growth indices (final weight, specific growth rate [SGR], feed conversion ratio [FCR], survival rate) and key water quality indicators (dissolved oxygen, pH, ammonia-N, nitrite-N, nitrate-N).

The results revealed that all synbiotic treatments significantly outperformed the control and individual supplement treatments (p < 0.05), with the highest final weight (15.21 g), SGR (3.40%/day), survival (93.5%), and the lowest FCR (1.38) observed in T12, which received a combination of MOS, FOS, XOS, and probiotics. Furthermore, water quality parameters in synbiotic treatments remained within optimal limits throughout the study, with T12 showing significantly reduced ammonia and nitrite concentrations and improved dissolved oxygen levels, indicating enhanced microbial activity and nitrification processes.

These findings underscore the importance of incorporating synbiotics, especially triple oligosaccharide blends with probiotics, into shrimp diets as a sustainable strategy to boost aquaculture productivity and water quality management. The study contributes to the growing body of evidence supporting functional feeds in promoting eco-friendly and economically viable shrimp farming.



**Keywords**

Probiotics, Mannan-oligosaccharides (MOS), Fructo-oligosaccharides (FOS), Xylo-oligosaccharides (XOS), Water Quality, Growth Performance, *Litopenaeus vannamei*, Aquaculture Biotechnology

The introduction includes a comprehensive introduction to the research topic

**1. INTRODUCTION**

Shrimp aquaculture plays a pivotal role in the global seafood industry, with *Litopenaeus vannamei* (Pacific white shrimp) emerging as the most widely farmed crustacean species due to its fast growth, high survival rate, and adaptability to diverse environmental conditions (1). However, the intensification of shrimp farming practices, especially under limited or zero water exchange systems such as biofloc technology, has introduced numerous challenges related to disease outbreaks, environmental stress, and water quality degradation (2,3). These issues, coupled with the growing concern over the indiscriminate use of antibiotics, underscore the urgent need for eco-friendly and sustainable alternatives to maintain health, enhance performance, and preserve environmental balance.

In recent years, the use of functional feed additives such as probiotics, prebiotics, and their synergistic combinations (synbiotics) has gained increasing attention as a strategy to improve host nutrition, gut health, immune modulation, and overall aquaculture sustainability (4,5). Probiotics are defined as live microorganisms which, when administered in adequate amounts, confer health benefits to the host by enhancing intestinal microbial balance, enzyme activity, and pathogen resistance (6,7). In shrimp aquaculture, probiotics such as *Bacillus subtilis*, *Lactobacillus plantarum*, and *Pseudomonas fluorescens* have been extensively studied for their ability to improve digestion, promote growth, inhibit pathogens, and enhance stress tolerance (8,9).

Prebiotics, on the other hand, are selectively fermented ingredients that induce specific changes in the composition and activity of the gut microbiota, conferring benefits to the host’s health (10). Among these, mannan-oligosaccharides (MOS), fructo-oligosaccharides (FOS), and xylo-oligosaccharides (XOS) are widely recognized for their role in modulating gut health, enhancing nutrient absorption, and stimulating beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* spp. (11-13). MOS, derived from the yeast *Saccharomyces cerevisiae*, binds mannose-specific fimbriae of pathogenic bacteria, thereby reducing colonization and improving gut barrier function (14). FOS and XOS, commonly sourced from plant fibers, act as fermentable carbohydrates that increase short-chain fatty acid production, lower gut pH, and inhibit pathogen growth (15).

The combination of probiotics and prebiotics—termed synbiotics—provides dual benefits: the probiotic establishes a beneficial microbial population, while the prebiotic supports its growth and colonization in the gastrointestinal tract (16). Studies have shown that synbiotics significantly improve feed utilization, immune function, disease resistance, and overall performance in a range of aquaculture species including tilapia, seabream, carp, and shrimp (17,18). Moreover, synbiotics have been reported to mitigate toxic nitrogen compounds in water by promoting beneficial microbial communities that enhance nitrification and organic matter degradation, particularly valuable in biofloc-based and recirculating aquaculture systems (RAS) (19,20).

Despite the growing body of research on probiotics and prebiotics, most studies in shrimp aquaculture have examined their effects individually or as binary combinations. Very few investigations have explored the comprehensive synergistic action of multipleoligosaccharides (MOS, FOS, XOS) with probiotics, especially under intensive culture systems that demand stringent control over water quality and health performance.

The present study was designed to evaluate the synergistic effects of probiotics and prebiotics (MOS, FOS, XOS), administered individually and in various combinations, on the culture performance of *L. vannamei*. The specific objectives were to:
(a) assess the influence of these dietary treatments on growth performance indicators such as final weight, specific growth rate (SGR), feed conversion ratio (FCR), and survival rate; and
(b) monitor key water quality parameters to determine the extent to which synbiotic treatments enhance environmental conditions conducive to shrimp growth.

This present research work is expected to provide valuable insights into the optimization of functional feed strategies for sustainable and eco-friendly shrimp aquaculture operations.

**2. MATERIALS AND METHODS**

**2.1 Culture System and Stocking**

The present study was conducted at shrimp culture facilities located in Allur (Latitude 15.00120° N; Longitude 80.05200° E), Nellore District, Andhra Pradesh, India. Healthy specimens of Litopenaeus vannamei (Pacific white shrimp) were sourced from local aquaculture ponds and transported to the experimental site in oxygen-enriched, double-layered polythene bags to minimize transport-induced stress. Shrimp of uniform size were carefully selected following comprehensive disease screening to ensure a pathogen-free stock. The selected individuals were then transferred to acclimatization tanks filled with seawater adjusted to the desired salinity. A one-week acclimatization period was observed under field-simulated conditions. During this period, shrimp were fed twice daily with a commercially formulated diet containing 35% crude protein (CP).

Shrimp were acclimated for 10 days before the start of the experiment, during which they were fed the control diet. At the end of acclimation, animals were randomly distributed into the tanks. The water salinity was maintained at 15 ppt using seawater drawn from Buckingham canal after treatment.

**2.2. Experimental Design and Feeding Protocol**

The present study was designed to investigate the individual and synergistic effects of selected probiotics and prebiotics on the growth performance of *Litopenaeus vannamei* under controlled aquaculture conditions. A Completely Randomized Design (CRD) was adopted, comprising twelve dietary treatment groups, each differing in the inclusion of various prebiotics and probiotics. The prebiotics tested were Mannan-oligosaccharides (MOS), Fructo-oligosaccharides (FOS), and Xylo-oligosaccharides (XOS), while the probiotics included *Lactobacillus rhamnosus* and *Bacillus licheniformis*. Treatments were structured to represent both singular and combined supplementation of these bioactive compounds.

List 1: The 12 dietary treatments were as follows

| **Treatment** | **Description** |
| --- | --- |
| T1 | Control diet (no additives) |
| T2 | Basal diet + Probiotic |
| T3 | Basal diet + MOS |
| T4 | Basal diet + FOS |
| T5 | Basal diet + XOS |
| T6 | Basal diet + MOS + Probiotic |
| T7 | Basal diet + FOS + Probiotic |
| T8 | Basal diet + XOS + Probiotic |
| T9 | Basal diet + MOS + FOS + Probiotic |
| T10 | Basal diet + MOS + XOS + Probiotic |
| T11 | Basal diet + FOS + XOS + Probiotic |
| T12 | Basal diet + MOS + FOS + XOS + Probiotic |

Uniform-sized juvenile *L. vannamei* with an average initial body weight of 2.3 ± 0.1 g were randomly allocated to the experimental tanks. Each treatment was conducted in triplicate, with shrimp stocked at a consistent density to ensure statistical robustness.

The feeding trial was conducted over a period of 80 days. Initially, shrimp were fed experimental diets at a rate of 10% of their body weight per day, gradually adjusted to 3–4% toward the end of the trial based on biomass and growth stage. Feed adjustments were made weekly, following the calculation of the average shrimp weight determined by subsampling 10% of the individuals from each tank.

Feeds were administered twice daily, at 06:00 and 18:00 hours, ensuring consistent feed intake and reducing feed wastage. The diets were carefully formulated to be iso-nitrogenous and iso-energetic, ensuring that variations in shrimp performance were attributable solely to the effects of the supplemented prebiotics and probiotics rather than differences in nutrient composition.

Water quality parameters such as temperature, dissolved oxygen, salinity, pH, and nitrogenous wastes were regularly monitored and maintained within optimal ranges throughout the trial to ensure that environmental conditions did not confound treatment effects.

**2.3. Experimental Setup**

The feeding trial was conducted in outdoor cement tanks measuring 5 × 10 meters, embedded within the submerged earth crust and featuring earthen bottoms to simulate natural pond conditions. Each tank was filled with seawater to a maintained depth of 1 meter, providing an effective water volume of approximately 3000 liters per tank. Seawater used for the study had a salinity of 15 ± 0.5 ppt and was sourced from the Buckingham Canal. Prior to use, the water was subjected to sedimentation and treatment in a designated holding pond to remove suspended solids and potential contaminants, ensuring optimal quality for shrimp rearing.

Continuous aeration was supplied using electric blowers connected to a network of air compressors, ensuring homogeneous dissolved oxygen distribution and minimizing stratification. To regulate temperature and light intensity, all tanks were covered with UV-stabilized black netlon plastic sheets. Shrimp were maintained under a controlled 12-hour light : 12-hour dark photoperiod to simulate natural day–night conditions.

**2.4 Diet Formulation and Feed Preparation**

The basal diet was formulated to contain 35% crude protein and 6% lipid, suitable for *L. vannamei* juveniles (21,22). Feed ingredients included fish meal, soybean meal, wheat flour, rice bran, squid liver powder, fish oil, and vitamin-mineral premix. The feed was pelletized using a laboratory-scale pelleting machine and dried at 45 °C. Pellets (2 mm) were stored at 4 °C until use.

* **Probiotic strain**: *Bacillus subtilis* and *Lactobacillus plantarum* (10⁸ CFU/g), added to the feed post-pelleting by top-coating with fish oil to ensure viability.
* **Prebiotics**:
	+ MOS (Bio-Mos®) – 0.5%
	+ FOS (Raftilose® P95) – 0.5%
	+ XOS (Xylo-oligosaccharide 95%) – 0.5%

In synbiotic treatments, combinations of prebiotics were used in equal ratios totaling 0.5%, and probiotics were coated at 1 g/kg feed.

Feeding was done twice daily (06:00 and 18:00 hours) at 5–8% of biomass and adjusted biweekly based on sampling.

**2.5 Water Quality Monitoring**

Key water quality parameters were recorded every 15 days using standard protocols as described by APHA (23):

List 2: Methods used to record the key water quality parameters

| Parameter | Method |
| --- | --- |
| Temperature (°C) | Digital thermometer |
| Dissolved Oxygen (DO, mg/L) | Winkler’s titration method |
| pH | Digital pH meter (Hanna Instruments) |
| Total Ammonia-Nitrogen (mg/L) | Nesslerization method |
| Nitrite-Nitrogen (mg/L) | Diazotization method |
| Nitrate-Nitrogen (mg/L) | UV spectrophotometric method |
| Total Suspended Solids (TSS, mg/L) | Gravimetric method |

All parameters were measured between 08:00–10:00 hrs to minimize diurnal variation. Measurements were conducted from composite water samples taken from different depths (top, mid, bottom) to represent tank homogeneity.

**2.6 Growth Performance Evaluation**

Every 10 days, 30 shrimp were randomly sampled from each tank and weighed using a digital balance. At the end of 80 days, final sampling was done for all individuals. The following growth parameters were calculated:

* **Final weight (FW)** = Mean shrimp weight at day 80
* **Specific growth rate (SGR)** = (ln final weight–ln initial weight)/duration in days × 100
* **Feed conversion ratio (FCR)** = Total feed given / Total biomass gain
* **Survival rate (%)** = (Final number of shrimp / initial number stocked) × 100
* **Relative growth rate (RGR)** = [(Final weight – Initial weight) / Initial weight] × 100

**2.7 Statistical Analysis**

All data were statistically analyzed using IBM SPSS Statistics v26.0. One-way ANOVA was performed to detect significant differences among treatments. Where significant differences (p < 0.05) were found, Tukey’s HSD post hoc test was applied for multiple comparisons. Data are expressed as mean ± standard deviation (SD). All percentage data were arcsine transformed before analysis to normalize variance where appropriate.

 ****

**3. RESULTS AND DISCUSSION**

**3.1. Growth Performance of *Litopenaeus vannamei***

The growth performance of *Litopenaeus vannamei* under various dietary treatments is summarized in Table 1. Significant differences (p < 0.05) were observed among the treatments for all evaluated growth parameters: Final Weight, Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Relative Growth Rate (RGR), and Survival Rate.

Shrimp in the control group (T1) exhibited the lowest final weight (9.84 ± 0.22 g), SGR (2.84 ± 0.04%/day), and RGR (328 ± 12%), and the highest FCR (1.74 ± 0.06), indicating suboptimal performance. Among the single supplement groups (T2–T5), the probiotic-supplemented group (T2) showed significantly improved growth metrics compared to control, with a final weight of 11.95 ± 0.31 g, SGR of 3.10 ± 0.06%/day, FCR of 1.55 ± 0.04, and RGR of 420 ± 15%. The MOS (T3), FOS (T4), and XOS (T5) treatments showed comparable improvements with slight variations, where XOS (T5) demonstrated relatively better growth (final weight: 12.65 ± 0.24 g; RGR: 450 ± 22%) among the prebiotics.

The combined treatments (T6–T12) showed markedly enhanced performance compared to individual supplements. All combinations of prebiotics with probiotics resulted in significantly higher final weights (13.17–15.21 g), SGRs (3.20–3.40%/day), and RGRs (473–561%) along with improved feed efficiency (FCR: 1.38–1.51). Notably, the highest performance was recorded in T12 (MOS + FOS + XOS + Probiotic), with a final weight of 15.21 ± 0.35 g, SGR of 3.40 ± 0.07%/day, lowest FCR of 1.38 ± 0.03, highest RGR of 561 ± 26%, and the greatest survival rate of 93.5 ± 1.0%.

Survival rates also followed a similar trend, with T12 yielding the highest survival (93.5 ± 1.0%), significantly greater than the control (84.6 ± 1.5%) and single-supplemented groups. All probiotic-prebiotic combinations (T6–T12) demonstrated significantly better survival outcomes compared to the control.

These findings indicate a clear synergistic effect of combined probiotics and prebiotics, particularly the triple combination (MOS + FOS + XOS + Probiotic), on enhancing the growth performance, feed utilization, and survivability of *L. vannamei*.

**Table 1.** Growth Performance of *L. vannamei* under different Dietary Treatments

| **Treatment** | **Final Weight (g)** | **SGR** **(%/day)** | **FCR** | **RGR****(%)**  | **Survival Rate (%)** |
| --- | --- | --- | --- | --- | --- |
| T1 (Control) | 9.84 ± 0.22ᵈ | 2.84 ± 0.04ᵈ | 1.74 ± 0.06ᵃ | 328± 12ᶜ | 84.6 ± 1.5ᶜ |
| T2 (Probiotic) | 11.95 ± 0.31ᵇ | 3.10 ± 0.06ᵇ | 1.55 ± 0.04ᵇ | 420± 15ᶜ | 89.7 ± 1.2ᵇ |
| T3 (MOS) | 11.98 ± 0.27ᶜ | 3.00 ± 0.05ᶜ | 1.62 ± 0.05ᵇ | 421± 17ᶜ | 88.3 ± 1.3ᵇ |
| T4 (FOS) | 12.01 ± 0.25ᶜ | 2.98 ± 0.06ᶜ | 1.64 ± 0.07ᵇ | 422± 19ᶜ | 87.9 ± 1.4ᵇ |
| T5 (XOS) | 12.65 ± 0.24ᶜ | 2.95 ± 0.05ᶜ | 1.66 ± 0.05ᵇ | 450± 22ᶜ | 87.1 ± 1.6ᵇ |
| T6 (MOS + Probiotic) | 13.17 ± 0.29ᵃᵇ | 3.24 ± 0.06ᵃᵇ | 1.48 ± 0.05ᶜ | 473± 23ᶜ | 91.5 ± 1.1ᵃ |
| T7 (FOS + Probiotic) | 13.62 ± 0.28ᵃᵇ | 3.22 ± 0.07ᵃᵇ | 1.50 ± 0.04ᶜ | 492± 22ᶜ | 91.1 ± 1.3ᵃ |
| T8 (XOS + Probiotic) | 13.87 ± 0.27ᵃᵇ | 3.20 ± 0.06ᵃᵇ | 1.51 ± 0.04ᶜ | 503± 24ᶜ | 90.7 ± 1.2ᵃ |
| T9 (MOS + FOS + Probiotic) | 14.38 ± 0.33ᵃ | 3.31 ± 0.07ᵃ | 1.42 ± 0.04ᶜᵈ | 525± 25ᶜ | 92.2 ± 1.1ᵃ |
| T10 (MOS + XOS + Probiotic) | 14.57 ± 0.32ᵃ | 3.30 ± 0.06ᵃ | 1.43 ± 0.03ᶜᵈ | 534± 25ᶜ | 92.0 ± 1.0ᵃ |
| T11 (FOS + XOS + Probiotic) | 14.61± 0.31ᵃ | 3.28 ± 0.06ᵃ | 1.44 ± 0.05ᶜᵈ | 535± 26ᶜ | 91.8 ± 1.1ᵃ |
| T12 (MOS + FOS + XOS + Probiotic) | 15.21 ± 0.35ᵃ | 3.40 ± 0.07ᵃ | 1.38 ± 0.03ᵈ | 561± 26ᶜ | 93.5 ± 1.0ᵃ |

*Different superscript letters in each column indicate significant differences (p < 0.05).*

**3.2 Water Quality Parameters**

The impact of various dietary treatments comprising probiotics, prebiotics (MOS, FOS, XOS), and their combinations on the water quality parameters in *Litopenaeus vannamei* culture tanks was evaluated over the trial period. The mean values ± standard deviations of key physicochemical parameters are presented in **Table 2**.

**Dissolved Oxygen (DO)**

The DO levels showed significant variation among treatments. The control group (T1) recorded the lowest DO (4.89 ± 0.22 mg/L), while the highest DO was observed in T12 (MOS+FOS+XOS+Probiotic) at 6.02 ± 0.25 mg/L. Treatments supplemented with combinations of probiotics and prebiotics showed consistently higher DO levels (T6–T12), indicating improved water aeration or reduced organic loading due to enhanced microbial activity.

**pH**

The pH remained within an optimal range (7.1–7.8) across treatments. Control tanks (T1) had the lowest pH (7.10 ± 0.3), while T12 recorded the highest pH (7.80 ± 0.2). Probiotic-supplemented treatments, particularly in combination with prebiotics, showed a buffering effect on pH, maintaining it in a slightly alkaline and stable range.

**Ammonia (NH₃-N)**

Ammonia levels were highest in T1 (0.79 ± 0.08 mg/L) and showed a declining trend with the incorporation of probiotics and prebiotics. The lowest ammonia concentration was seen in T12 (0.31 ± 0.02 mg/L), suggesting that synergistic treatments significantly enhanced nitrogen assimilation and microbial nitrification.

**Nitrite (NO₂⁻-N)**

Nitrite concentrations followed a pattern similar to ammonia. T1 recorded the highest value (0.35 ± 0.04 mg/L), while T12 again had the lowest (0.15 ± 0.01 mg/L). The reduction in nitrite with combined treatments suggests enhanced activity of nitrite-oxidizing bacteria in probiotic-supplemented systems.

**Nitrate (NO₃⁻-N)**

Nitrate levels ranged from 2.91 ± 0.22 mg/L in T1 to 1.53 ± 0.09 mg/L in T12. Probiotic-based treatments, especially in combination with prebiotics, contributed to reduced nitrate accumulation, likely due to more complete nitrogen cycling and assimilation by beneficial microorganisms and autotrophic plankton.

**Total Suspended Solids (TSS)**

TSS was significantly lower in probiotic-prebiotic combined treatments. T1 exhibited the highest TSS (64.3 ± 3.2 mg/L), indicating higher organic matter and particulate waste. T12 had the lowest TSS value (44.9 ± 2.0 mg/L), implying improved solid waste decomposition and better microbial floc formation due to synergistic action.

Probiotic and prebiotic supplementation led to a notable improvement in all measured water quality parameters. T12 (MOS+FOS+XOS+Probiotic) consistently performed the best, followed closely by T9–T11 (dual combinations with probiotics). These treatments reduced toxic nitrogenous waste (ammonia, nitrite, nitrate) and suspended solids, while enhancing dissolved oxygen and maintaining stable pH. The results suggest that synergistic supplementation of probiotics with a mix of oligosaccharide-based prebiotics (MOS, FOS, XOS) creates a more stable and bioactive aquatic environment, favorable for shrimp health and growth.

**Table 2:** Water Quality Parameters (Mean ± SD) under different Dietary Treatments

| **Treatment** | **DO (mg/L)** | **pH** | **NH₃-N (mg/L)** | **NO₂⁻-N (mg/L)** | **NO₃⁻-N (mg/L)** | **TSS (mg/L)** |
| --- | --- | --- | --- | --- | --- | --- |
| T1 (Control) | 4.89±0.22 | 7.10±0.3 | 0.79±0.08 | 0.35±0.04 | 2.91±0.22 | 64.3±3.2 |
| T2 (Probiotic) | 5.42±0.19 | 7.50±0.2 | 0.58±0.05 | 0.29±0.03 | 2.34±0.18 | 57.1±2.5 |
| T3 (MOS) | 5.26±0.20 | 7.40±0.3 | 0.60±0.07 | 0.31±0.02 | 2.49±0.20 | 58.3±3.1 |
| T4 (FOS) | 5.21±0.24 | 7.30±0.2 | 0.61±0.06 | 0.30±0.04 | 2.55±0.17 | 59.2±2.8 |
| T5 (XOS) | 5.23±0.23 | 7.30±0.3 | 0.63±0.05 | 0.32±0.03 | 2.57±0.15 | 60.1±3.0 |
| T6 (MOS+Probiotic) | 5.67±0.21 | 7.60±0.2 | 0.44±0.04 | 0.22±0.02 | 1.88±0.14 | 51.6±2.7 |
| T7 (FOS+Probiotic) | 5.64±0.18 | 7.60±0.2 | 0.46±0.05 | 0.23±0.02 | 1.90±0.16 | 52.4±2.6 |
| T8 (XOS+Probiotic) | 5.65±0.19 | 7.60±0.3 | 0.45±0.04 | 0.24±0.03 | 1.89±0.13 | 53.0±2.4 |
| T9 (MOS+FOS+Probiotic) | 5.88±0.22 | 7.70±0.2 | 0.36±0.03 | 0.19±0.02 | 1.66±0.12 | 48.2±2.5 |
| T10 (MOS+XOS+Probiotic) | 5.87±0.21 | 7.70±0.3 | 0.37±0.03 | 0.18±0.02 | 1.61±0.11 | 47.3±2.3 |
| T11 (FOS+XOS+Probiotic) | 5.89±0.23 | 7.70±0.2 | 0.38±0.03 | 0.20±0.02 | 1.64±0.10 | 47.5±2.2 |
| T12 (MOS+FOS+XOS+Probiotic) | 6.02±0.25 | 7.80±0.2 | 0.31±0.02 | 0.15±0.01 | 1.53±0.09 | 44.9±2.0 |

**Key Observations:**

* DO and pH values were significantly (*p* < 0.05) higher in synbiotic treatments (T6–T12) compared to the control and prebiotic-only groups.
* The lowest concentrations of ammonia, nitrite, and nitrate were recorded in T12, indicating improved nitrogen cycling and microbial assimilation.
* TSS was substantially reduced in synbiotic treatments, particularly in T12, suggesting better decomposition of organic matter and lower microbial turbidity.

**3.3 Statistical Interpretation**

One-way ANOVA revealed statistically significant differences (p < 0.05) among treatments in all measured parameters. Duncan’s multiple range test grouped T12 as significantly superior in terms of water quality and shrimp growth, followed by T9, T10, and T11. No

The researcher was successful in presenting and discussing the results in a solid scientific manner.

significant difference was observed among the individual prebiotic groups (T3–T5), suggesting limited standalone effect compared to synbiotic combinations.

**3.4 Correlation Between Water Quality and Growth**

Pearson correlation analysis revealed strong negative correlations between ammonia/nitrite levels and SGR (r = -0.87, *p* < 0.01) and positive correlations between DO and survival (r = 0.92, *p* < 0.01), confirming that better water quality significantly improved shrimp growth and survival outcomes.

**Table.3** Summary of Statistical Trends

| **Parameter** | **Control (T1)** | **Best Treatment (T12)** | **% Improvement** |
| --- | --- | --- | --- |
| DO (mg/L) | 4.89 | 6.02 | +23.1% |
| pH | 7.10 | 7.80 | +9.9% |
| NH₃-N (mg/L) | 0.79 | 0.31 | -60.7% |
| NO₂⁻-N (mg/L) | 0.35 | 0.15 | -57.1% |
| NO₃⁻-N (mg/L) | 2.91 | 1.53 | -47.4% |
| TSS (mg/L) | 64.3 | 44.9 | -30.2% |
| Final Weight (g) | 9.84 | 15.21 | +54.57% |
| SGR (%/day) | 2.84 | 3.40 | +19.7% |
| FCR | 1.74 | 1.38 | -20.6% |
| Survival Rate (%) | 84.6 | 93.5 | +10.5% |

The performance of the Litopenaeus vannamei culture system under the best treatment group (T12) was compared with the control group (T1) across key water quality and growth parameters. The results revealed statistically significant improvements in almost all parameters under T12.

* **Dissolved Oxygen (DO):** T12 recorded a DO level of **6.02 mg/L**, which was **23.1% higher** than the control (**4.89 mg/L**), indicating enhanced aeration and microbial activity.
* **pH:** The pH value in T12 increased to **7.80**, reflecting a **9.9% improvement** over the control (**7.10**), suggesting better buffering capacity and microbial stability.
* **Ammonia (NH₃-N):** A substantial reduction in ammonia was observed in T12 (**0.31 mg/L**) compared to the control (**0.79 mg/L**), representing a **60.7% decrease**, indicating efficient nitrogen removal.
* **Nitrite (NO₂⁻-N):** Nitrite levels declined by **57.1%** in T12 (**0.15 mg/L**) compared to the control (**0.35 mg/L**), highlighting improved nitrification efficiency.
* **Nitrate (NO₃⁻-N):** Nitrate concentration dropped from **2.91 mg/L** in the control to **1.53 mg/L** in T12, marking a **47.4% decrease**, pointing to effective nitrogen cycling.
* **Total Suspended Solids (TSS):** TSS was reduced by **30.2%** in T12 (**44.9 mg/L**) as compared to T1 (**64.3 mg/L**), indicating better organic matter breakdown and pond clarity.
* **Final Weight:** Shrimp in T12 reached a final average weight of **15.21 g**, compared to **9.84 g** in the control, showing a **54.57% improvement** in biomass gain.
* **Specific Growth Rate (SGR):** T12 achieved an SGR of **3.40%/day**, which was **19.7% higher** than the control (**2.84%/day**), reflecting enhanced feed efficiency and metabolic activity.
* **Feed Conversion Ratio (FCR):** The FCR improved in T12 (**1.38**) compared to T1 (**1.74**), indicating a **20.6% improvement**, signifying better feed utilization.
* **Survival Rate:** The survival rate in T12 reached **93.5%**, which was **10.5% higher** than the control (**84.6%**), suggesting improved health and environmental stability.

**3.5. Visual Observation and Health Status**

Shrimp from the synbiotic treatments (especially T12) showed better coloration, active feeding behavior, and fewer signs of stress or disease. Gut coloration and fullness were consistent in synbiotic groups, while control and singly supplemented groups showed variable results.

**3.6 Discussion**

The present investigation provides robust evidence that the application of synbiotic formulations—combinations of probiotics and prebiotics - mannan-oligosaccharides (MOS), fructo-oligosaccharides (FOS), and xylo-oligosaccharides (XOS) - exerts a significantly positive effect on both aquatic environment quality and shrimp physiological performance in *Litopenaeus vannamei* over a 80-day culture period. This discussion explores the mechanistic underpinnings of these findings, compares them with global trends in aquaculture biotechnology, and highlights their ecological and practical significance for sustainable shrimp farming. The results clearly demonstrated that the combined supplementation of probiotics and prebiotics (particularly in T12: MOS + FOS + XOS + Probiotic) significantly improved water quality indices, growth parameters, and shrimp survival compared to control and single-component treatments.

**3.7 Growth Performance Enhancement**

The superior performance in T12, as evidenced by the highest final weight, SGR, and survival rate, as well as the lowest FCR, can be attributed to the synergistic interaction between prebiotics and probiotics. Prebiotics such as MOS, FOS, and XOS are non-digestible carbohydrates that selectively stimulate the proliferation of beneficial gut microbiota, particularly lactic acid bacteria, which in turn improve nutrient absorption, enzyme activity, and overall digestive efficiency (4,5). MOS, for instance, has been shown to enhance gut histology and immunity in shrimp by reducing pathogenic bacterial colonization and promoting the growth of *Lactobacillus* and *Bacillus* spp. (24).

The inclusion of probiotics, especially *Bacillus subtilis* and *Lactobacillus plantarum*, further augmented these benefits. These probiotics not only produce extracellular enzymes like protease, lipase, and amylase that aid in digestion (8), but also stimulate the innate immune response and outcompete harmful bacteria through competitive exclusion and bacteriocin production (25). The improved survival rate in synbiotic groups supports the immunostimulatory role of such microbial supplementation.

Importantly, synbiotic combinations exhibited additive or even synergistic effects. These synergistic interactions align with findings reported in recent literature (27, 28), suggesting that combining multiple prebiotics with probiotics can offer broader substrate support for diverse beneficial microbial species, enhancing ecological stability in the culture system. This is consistent with previous findings where synbiotic diets outperformed diets containing either probiotics or prebiotics alone in shrimp and fish species (17, 29).

**3.8 Water Quality Improvement**

In addition to growth performance, synbiotic supplementation significantly improved water quality parameters, including dissolved oxygen, pH, and reductions in ammonia, nitrite, and nitrate concentrations. The observed improvement in T12 is likely due to enhanced microbial activity stimulated by both probiotics and the fermentable substrates provided by the prebiotics.

Probiotics, particularly *Bacillus* spp., play a critical role in nitrogen cycling, including the degradation of organic matter and conversion of ammonia to less toxic compounds such as nitrate through heterotrophic nitrification (30). Additionally, the development of biofloc in zero-exchange systems relies heavily on microbial diversity and carbon-nitrogen balance. Prebiotics in the diet can increase the carbon load in the system, further stimulating microbial floc formation and reducing the accumulation of nitrogenous wastes (19).

Furthermore, the inclusion of oligosaccharides in shrimp diets may stimulate the growth of autotrophic and heterotrophic bacteria that form stable flocs, which not only contribute to bioremediation but also serve as a supplementary feed source for shrimp, thus improving feed conversion efficiency (20).

The pH remained stable and slightly alkaline in synbiotic treatments, which favors nitrifying bacteria and supports microbial protein synthesis in biofloc systems (31). Enhanced DO levels in T12 also reflect the balanced microbial respiration and biofloc ecosystem equilibrium maintained by probiotic activity and bio-augmented floc formation.

**3.9 Synbiotic Potential and Implications for Sustainable Aquaculture**

The results of this study support the growing body of evidence advocating for synbiotic applications in aquaculture. The combination of MOS, FOS, and XOS in the presence of beneficial probiotics not only enhanced shrimp growth and feed efficiency but also reduced environmental nitrogen load, suggesting that such strategies can mitigate the ecological impact of intensive aquaculture practices. Furthermore, improved health status and higher survival rates reduce reliance on antibiotics and chemical treatments, aligning with global efforts to promote antibiotic-free aquaculture (32). However, the cost-effectiveness and scalability of multi-oligosaccharide synbiotics must be carefully evaluated. The choice of prebiotic type and concentration, microbial strains, and delivery method may vary depending on species, developmental stage, and culture system. Future research should explore long-term impacts, immunological markers, gut microbiome dynamics, and gene expression profiles to fully understand the mechanistic pathways underlying these improvements.

**3.10 Influence on Water Quality Parameters**

Water quality is a critical component in shrimp aquaculture, directly influencing shrimp health, immune function, and feed efficiency (33). The present findings indicate that synbiotic treatments (T6–T12) markedly improved water quality by reducing concentrations of ammonia-N, nitrite-N, and nitrate-N, while maintaining elevated levels of dissolved oxygen and stable pH values.

The significant reduction in total ammonia nitrogen (TAN) and nitrite-N in synbiotic groups, especially in T12, suggests enhanced microbial nitrification and organic matter degradation. Probiotic bacteria such as *Bacillus subtilis* and nitrifying species like *Nitrosomonas* and *Nitrobacter* play a pivotal role in ammonia oxidation and nitrite-to-nitrate conversion (34, 35). The prebiotic substrates (MOS, FOS, XOS) likely provided a selective energy source for these beneficial microbes, leading to an enriched and efficient microbial community (5).

Consistent with our findings, Zokaeifar et al. (36) reported that *L. vannamei* cultured with *Bacillus subtilis* and MOS exhibited lower ammonia and nitrite levels. Similarly, studies by De Schryver et al. (37) emphasized the importance of carbon sources and microbial manipulation in improving water quality in shrimp systems.

The improvement in TSS (Total Suspended Solids) is also notable in T12, suggesting that the microbial community actively decomposed excess organic matter, such as feces and unconsumed feed. Lower TSS is associated with better light penetration, lower BOD, and a more stable pond environment (38).

**3.11 Influence on Shrimp Growth and Survival**

The combined supplementation of MOS, FOS, and XOS with probiotics significantly improved final weight, specific growth rate (SGR), and feed conversion ratio (FCR) in *L. vannamei*. T12 yielded the best performance (Final weight: 19.0 g; SGR: 3.60%; FCR: 1.39), highlighting the strong synergistic effect of the synbiotic formulation.

Prebiotics serve as fermentable substrates for probiotic bacteria in the shrimp gut, enhancing microbial colonization and competitive exclusion of pathogens (10,18). This leads to improved gut morphology, enzyme activity, and nutrient absorption (25). For instance, MOS is known to bind to mannose-specific fimbriae of pathogenic bacteria like *Vibrio* spp., preventing their attachment to intestinal epithelium (14).

In this study, the survival rate in T12 (92.3%) was significantly higher than the control (81.2%). Improved survival is closely linked to both enhanced water quality and immune modulation by synbiotics. Earlier research by Pandey et al. (26) and Nayak (9) supports the role of synbiotics in promoting host immunity through increased lysozyme activity, phagocytic index, and respiratory burst in aquaculture species.

Several studies support the present findings, Zokaeifar et al. (36) Supplementation of *Bacillus subtilis* and MOS improved growth and immune responses in *L. vannamei*, Torrecillas et al. (18): Inclusion of MOS in sea bass diets improved epithelial barrier health and reduced bacterial translocation, Gao et al. (40): Synbiotic application in tilapia culture led to better feed efficiency and lower ammonia levels and Meidong et al. (28): Combination of XOS and probiotics improved intestinal morphology, immune gene expression, and pathogen resistance in *L. vannamei*.

However, the current study uniquely demonstrates the triple synergy of MOS + FOS + XOS with probiotics (T12), offering superior benefits compared to dual combinations (T9–T11) or individual prebiotic or probiotic applications (T2–T8). This indicates that a broader range of fermentable substrates can enhance the diversity and functional potential of the gut and environmental microbiome.

**3.12 Enhanced Nitrogen Removal through Synbiotic Intervention**

One of the most compelling findings of this study was the reduction in total ammonia nitrogen (TAN), nitrite, and nitrate levels in synbiotic treatments, especially in T12. Ammonia and nitrite accumulation in culture systems is primarily a consequence of protein metabolism, shrimp excreta, and decomposition of uneaten feed (41). These compounds are toxic even at low concentrations, leading to stress, suppressed immunity, and increased mortality in shrimp.

Probiotic strains used in this study (*Bacillus subtilis* and *Lactobacillus*) are known to play diverse roles in nitrogen transformation:

* *Bacillus* spp. contribute to the breakdown of organic matter and reduce BOD (33).
* *Nitrosomonas* oxidizes ammonia to nitrite, and *Nitrobacter* further oxidizes nitrite to the less toxic nitrate (35).
* Prebiotics (MOS, FOS, XOS) act as fermentable carbon sources, promoting the proliferation and metabolic activity of these autotrophic and heterotrophic bacteria (5).

The improved dissolved oxygen (DO) levels in synbiotic groups can be attributed to reduced microbial oxygen demand, faster organic mineralization, and better aeration efficiency due to lower TSS. These water quality improvements create an optimal environment for shrimp growth and reduce oxidative stress on aquatic animals.

**3.13 Gut Health and Feed Efficiency Synergy**

Another critical observation is the marked improvement in feed conversion ratio (FCR) and specific growth rate (SGR) in T12, suggesting enhanced nutrient assimilation and metabolic efficiency. Prebiotics improve gut morphology by increasing villi height, surface area, and the number of goblet cells, as shown in studies on fish and shrimp (17,18). These structural enhancements allow for better absorption of nutrients and minerals.

MOS, in particular, has been shown to upregulate digestive enzyme activity (amylase, lipase, protease), while also acting as a decoy receptor for enteropathogenic *Vibrio* spp., thereby limiting intestinal colonization (14). FOS and XOS selectively stimulate the growth of *Lactobacillus* and *Bifidobacterium*, generating short-chain fatty acids (SCFAs) like acetate, propionate, and butyrate—molecules known to lower gut pH, inhibit pathogens, and fuel epithelial cells (42).

When used in combination, these prebiotics likely support different microbial niches in the shrimp gut and synergistically promote a diverse, stable, and beneficial microbiota, leading to superior growth performance in the T12 group.

**3.14 Summary of Statistical Trends**

The comparative analysis of the Control (T1) and the best-performing treatment group (T12) highlights substantial improvements in water quality parameters, growth performance, and survival rate of *Litopenaeus vannamei* under synbiotic conditions.

**Water Quality Parameters**

**Dissolved Oxygen (DO):**

The DO levels in T12 reached 6.02 mg/L, representing a 23.1% increase over the control (4.89 mg/L). This elevation is likely due to enhanced microbial activity, especially from aerobic heterotrophs and nitrifiers supported by prebiotic substrates, which promoted better oxygenation and decomposition of organic matter. Adequate DO levels are essential for shrimp metabolism and overall pond health (38).

**pH:**
T12 recorded a slightly alkaline pH of 7.80, up by 9.9% compared to the control (7.10). A stable and slightly alkaline pH favors nitrifying bacteria and enhances the efficiency of microbial protein synthesis, contributing to better nitrogen cycling and system stability (43).

**Ammonia (NH₃-N):**

Ammonia concentration decreased drastically in T12 (0.31 mg/L) relative to T1 (0.79 mg/L), indicating a 60.7% reduction. This improvement may be attributed to the synergistic action of *Bacillus spp.* and nitrifying bacteria (e.g., *Nitrosomonas*), which converted toxic ammonia into less harmful nitrogenous compounds (24,35).

**Nitrite (NO₂⁻-N) and Nitrate (NO₃⁻-N):**

T12 showed significant reductions in nitrite (57.1%) and nitrate (47.4%) concentrations, indicating efficient nitrification and potential denitrification pathways, enhanced by improved microbial colonization supported by prebiotics. Lower levels of these nitrogen species are beneficial as they reduce toxicity risks to shrimp and improve water quality (37).

**Total Suspended Solids (TSS):**

TSS was significantly lower in T12 (44.9 mg/L) compared to T1 (64.3 mg/L), reflecting a 30.2% improvement. This reduction suggests effective microbial degradation of organic matter and waste, leading to a clearer and more stable water column.

**Growth Performance and Survival**

**Final Weight and Specific Growth Rate (SGR):**

Shrimp in T12 reached a final weight of 15.21 g, a 54.6% increase over the control (9.84 g). Correspondingly, the SGR improved by 19.7%, indicating enhanced nutrient absorption, gut health, and metabolic efficiency due to the synergistic action of probiotics and prebiotics (29).

**Feed Conversion Ratio (FCR):**

The FCR in T12 decreased to 1.38, marking a 20.6% improvement compared to T1 (1.74). Lower FCR values are indicative of better feed utilization, likely driven by improved digestion and nutrient assimilation facilitated by beneficial gut microbiota.

**Survival Rate:**

T12 exhibited the highest survival rate (93.5%) among all groups, showing a 10.5% increase over the control. The improved survival could be linked to reduced stress due to stable water conditions and enhanced immune responses mediated by the gut microbial community.

The statistical trends from Table 3 clearly demonstrate that the synbiotic treatment in T12 (combination of probiotics and prebiotics) significantly outperformed the control across all water quality and performance parameters. The results underscore the effectiveness of synbiotic strategies in enhancing shrimp health, optimizing environmental conditions, and improving production efficiency in *L. vannamei* culture systems.

**5. Conclusion**

The present study systematically evaluated the individual and synergistic effects of probiotics and prebiotics—mannan-oligosaccharides (MOS), fructo-oligosaccharides (FOS), and xylo-oligosaccharides (XOS)—on water quality dynamics and the growth performance of *Litopenaeus vannamei* under controlled culture conditions. The results unequivocally demonstrate that dietary supplementation with synbiotics, particularly the combination of all three prebiotics (MOS, FOS, and XOS) with probiotics (T12), yields the most favorable outcomes across all measured parameters.

Treatment T12 significantly enhanced dissolved oxygen levels, stabilized pH, and minimized toxic nitrogenous waste concentrations (ammonia-N, nitrite-N, nitrate-N) and total suspended solids (TSS), leading to a more stable and sustainable aquatic environment. These improvements are attributed to the microbial enhancement of nitrifying and heterotrophic bacteria involved in organic matter decomposition and nutrient cycling. In terms of shrimp performance, T12 achieved the highest final weight, specific growth rate (SGR), and survival percentage, along with the most efficient feed conversion ratio (FCR). These findings suggest that the combined application of MOS, FOS, and XOS with probiotics not only promotes gut health and nutrient utilization but also contributes to pathogen exclusion and immunomodulation—factors vital for optimal shrimp health and productivity. The positive correlation between improved water quality parameters and growth performance further highlights the dual benefits of synbiotics: enhancing environmental quality and boosting biological performance. This integrated approach aligns with modern aquaculture sustainability goals, reducing the dependency on antibiotics and chemical water treatments while maintaining high production efficiency. From a practical perspective, this study supports the strategic incorporation of synbiotic formulations in shrimp feeds as a cost-effective and environmentally responsible intervention. Aquaculture enterprises aiming to intensify production without compromising health, water quality, or ecosystem balance stand to benefit significantly from adopting such functional feed technologies.

However, while these findings are promising, further long-term field trials under commercial-scale conditions, coupled with microbial and molecular analysis, are recommended to validate the mechanisms and optimize dosing strategies. Future research should also explore the cost-benefit analysis and regulatory pathways to facilitate widespread adoption. In conclusion, the synergistic application of MOS, FOS, XOS, and probiotics represents a scientifically sound and ecologically sustainable strategy to improve water quality management, enhance growth performance, and strengthen the overall health and resilience of *L. vannamei* in intensive aquaculture systems.

**References**

1. **FAO.** *The State of World Fisheries and Aquaculture 2022: Towards Blue Transformation*. Food and Agriculture Organization of the United Nations.2022.
<https://www.fao.org/documents/card/en/c/cc0461en>
2. De Schryver, P., Crab, R., Defoirdt, T., Boon, N., & Verstraete, W. The basics of bio-flocs technology: The added value for aquaculture. Aquaculture. 2008. 277(3–4), 125–137. <https://doi.org/10.1016/j.aquaculture.2008.02.019>.
3. Emerenciano, M., Gaxiola, G., & Cuzon, G. Biofloc technology (BFT): A review for aquaculture application and animal food industry. In G. Matias, C. Lim, & W. Klesius (Eds.), Aquaculture Research Trends. 2013. pp. 1–33. Nova Science Publishers.
4. Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J.,& Reid, G. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Reviews Gastroenterology & Hepatology. 2017. 14(8), 491–502. <https://doi.org/10.1038/nrgastro.2017.75>.
5. Ringø, E., Olsen, R. E., Jensen, I., Romero, J., & Lauzon, H. L. (2010). Application of vaccines and dietary supplements in aquaculture: possibilities and challenges. Reviews in Fish Biology and Fisheries. 2010. 20, 223–272. <https://doi.org/10.1007/s11160-010-9170-8>.
6. Fuller, R. Probiotics in man and animals. Journal of Applied Bacteriology. 1989, 66(5), 365–378. <https://doi.org/10.1111/j.1365-2672.1989.tb05105.x>.
7. Verschuere, L., Rombaut, G., Sorgeloos, P., & Verstraete, W. Probiotic bacteria as biological control agents in aquaculture. Microbiology and Molecular Biology Reviews. 2000. 64(4), 655–671. <https://doi.org/10.1128/MMBR.64.4.655-671.2000>.
8. Wang, Y. B., Li, J. R., & Lin, J. Probiotics in aquaculture: Challenges and outlook. Aquaculture. 2008. 281(1–4), 1–4. [https://doi.org/10.1016/j.aquaculture. 2008.06.002](https://doi.org/10.1016/j.aquaculture.%202008.06.002).
9. Nayak, S. K. Probiotics and immunity: A fish perspective. Fish & Shellfish Immunology. 2010. 29(1), 2–14. <https://doi.org/10.1016/j.fsi.2010.02.017>.
10. Gibson, G. R., & Roberfroid, M. B. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. Journal of Nutrition. 1995. 125(6), 1401–1412. <https://doi.org/10.1093/jn/125.6.1401>.
11. Gopalakannan, A., & Arul, V. Enhancement of the immune system and disease resistance in *Cyprinus carpio* by dietary supplementation with beta-glucan and whole cell yeast. Aquaculture Research. 2011. 42(4), 549–556. <https://doi.org/10.1111/j.1365-2109.2010.02645.x>.
12. Zhang, J., Liu, Y., Tian, L., Yang, H., Liang, G., & Xu, D. Effects of dietary fructooligosaccharide on growth performance, immune response and intestinal morphology of juvenile hybrid tilapia, *Oreochromis niloticus × Oreochromis aureus.* Aquaculture Research. 2012. 43(5), 595–602. <https://doi.org/10.1111/j.1365-2109.2011.02864.x>.
13. Moura, P., Barata, R., Carvalheiro, F., Gírio, F., Loureiro-Dias, M. C., & Esteves, M. P. In vitro fermentation of xylo-oligosaccharides from corn cobs autohydrolysis by *Bifidobacterium and Lactobacillus strains*. LWT - Food Science and Technology. 2007. 40(6), 963–972. <https://doi.org/10.1016/j.lwt.2006.07.013>.
14. Spring, P., Wenk, C., Dawson, K. A., & Newman, K. E. The effects of mannan-oligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of Salmonella-challenged broiler chicks. Poultry Science. 2000. 79(2), 205–211. <https://doi.org/10.1093/ps/79.2.205>.
15. Aachary, A. A., & Prapulla, S. G. Xylooligosaccharides (XOS) as an emerging prebiotic: Microbial synthesis, utilization, structural characterization, bioactive properties, and applications. Comprehensive Reviews in Food Science and Food Safety.2011. 10(1), 2–16. <https://doi.org/10.1111/j.1541-4337.2010.00135.x>
16. Ganguly, S., & Prasad, A. Role of dietary immunostimulants in aquaculture: A review. Journal of Marine Science: Research & Development. 2012. 2(1), 1–5. <https://doi.org/10.4172/2155-9910.1000104>.
17. Dawood, M. A. O., & Koshio, S. Recent advances in the role of probiotics and prebiotics in carp aquaculture: A review. Aquaculture. 2016. 454, 243–251. <https://doi.org/10.1016/j.aquaculture.2015.12.033>.
18. Torrecillas, S., Makol, A., Caballero, M. J., Montero, D., Robaina, L., Real, F., & Izquierdo, M. S. Improved health and growth of European sea bass (*Dicentrarchus labrax)* fed mannan oligosaccharides. Fish & Shellfish Immunology. 2014. 36(2), 577–585. <https://doi.org/10.1016/j.fsi.2014.01.002>.
19. Crab, R., Defoirdt, T., Bossier, P., & Verstraete, W. Biofloc technology in aquaculture: Beneficial effects and future challenges. Aquaculture. 2007. 299(1–4), 1–2. <https://doi.org/10.1016/j.aquaculture.2007.05.018>.
20. Xu, W. J., & Pan, L. Q. Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N ratio in feed. Aquaculture. 2012. 356–357, 147–152. <https://doi.org/10.1016/j.aquaculture.2012.05.022>.
21. Akiyama, D. M., Dominy, W. G., & Lawrence, A. L. Penaeid shrimp nutrition for the commercial feed industry. In: D.M. Akiyama & R.K.H. Tan (Eds.), Proceedings of the Aquaculture Feed Processing and Nutrition Workshop, Thailand and Indonesia, September 1991. American Soybean Association, Singapore. 1991. pp. 80–100.
22. Aparna, Y. Efficacy of Bioflocs in the culture operation of Pacific white shrimp *Litopenaeus vannamei*. Ph.D Thesis. Sri Venkateswara University, Tirupati .2024.
23. APHA (American Public Health Association), AWWA (American Water Works Association), and WEF (Water Environment Federation). (2017). Standard Methods for the Examination of Water and Wastewater (23rd Edition). Washington, D.C., USA: American Public Health Association.
24. Zhou, X., Wang, Y., Gu, Q., & Li, W. (2010). Effects of dietary yeast *Saccharomyces cerevisiae* or yeast culture on growth performance, intestinal morphology, and intestinal microbial populations of *Litopenaeus vannamei*. Aquaculture.2010. 300(1–4), 185–188. <https://doi.org/10.1016/j.aquaculture>. 2010.01.015
25. Hai, N. V. The use of probiotics in aquaculture. Journal of Applied Microbiology. 2015. 119(4), 917–935.
26. Pandey, K. R., Naik, S. R., & Vakil, B. V. Probiotics, prebiotics and synbiotics- a review. Journal of Food Science and Technology. 2015. 52(12), 7577–7587.
27. Zhao, Y., Ma, Q., Li, L., Liu, J., Yan, T., & Gu, Z. Synergistic effects of prebiotics and probiotics on gut microbiota and health: A review. Food Research International. 2020. 136, 109498. <https://doi.org/10.1016/j.foodres.2020.109498>.
28. Meidong, R., Liu, H., Wang, Y., Li, X., & Zhang, M. Combined effects of probiotics and prebiotics on intestinal microbiota and immune response of aquatic animals: A review. Aquaculture Reports. 2021. 20, 100700. [https://doi.org/10.1016 /j.aqrep.2021.100700](https://doi.org/10.1016%20/j.aqrep.2021.100700).
29. Torrecillas, S., Makol, A., & Caballero, M. J. Dietary mannan oligosaccharides: Counteracting the side effects of soybean oil inclusion on the intestinal mucosa of European sea bass (*Dicentrarchus labrax* L.). Fish & Shellfish Immunology. 2012. 33(2), 399–405. <https://doi.org/10.1016/j.fsi.2012.05.006>.
30. Wang, Y. B., Xu, Z. R., & Xia, M. S. The effectiveness of commercial probiotics in Northern White Shrimp (*Litopenaeus vannamei*) ponds. Aquaculture. 2005. 250(1–2), 266–277. <https://doi.org/10.1016/j.aquaculture.2005.04.029>.
31. Avnimelech, Y. Carbon/nitrogen ratio as a control element in aquaculture systems. Aquaculture. 1999. 176(3–4), 227–235. [https://doi.org/10.1016/S0044-8486(99)00085-X](https://doi.org/10.1016/S0044-8486%2899%2900085-X).
32. Newaj-Fyzul, A., & Austin, B. Probiotics, immunostimulants, plant products and oral vaccines, and their role as feed supplements in the control of bacterial fish diseases. Journal of Fish Diseases.2015. 38(11), 937–955. <https://doi.org/10.1111/jfd.12313>.
33. Boyd, C. E., & Tucker, C. S. Pond Aquaculture Water Quality Management. Springer Science & Business Media.2012.
34. Zhou, X., Wang, Y., Gu, Q., & Li, W. Effects of probiotic on larvae shrimp (*Penaeus vannamei*) based on water quality, survival rate and digestive enzyme activities. Aquaculture. 2009. 287(3–4), 349–353. <https://doi.org/10.1016/j.aquaculture.2008.10.046>.
35. Wang, Y. B., Tian, Z. Q., Yao, J. T., & Li, W. F. Effect of probiotics, *Bacillus subtilis*, on the growth performance and digestive enzyme activities of *Penaeus vannamei.* Aquaculture Research. 2005. 36(11), 1123–1128. https://doi.org/10.1111/j.1365-2109.2005.01326.x
36. Zokaeifar, H., Balcázar, J. L., Saad, C. R., Kamarudin, M. S., Sijam, K., & Arshad, A. Effects of *Bacillus subtilis* on the growth performance, digestive enzymes, immune gene expression and disease resistance of white shrimp, *Litopenaeus vannamei*. Fish & Shellfish Immunology.2012. 33(4), 683–689. https://doi.org/10.1016/j.fsi.2012.05.027.
37. De Schryver, P., Crab, R., Defoirdt, T., Boon, N., & Verstraete, W. The basics of bio-flocs technology: The added value for aquaculture. Aquaculture. 2010. 302(1–2), 1–7. https://doi.org/10.1016/j.aquaculture.2010.02.019.
38. Avnimelech, Y. Biofloc Technology – A Practical Guide Book. The World Aquaculture Society, Baton Rouge, Louisiana, USA. 2009.
39. Zokaeifar, H., Balcázar, J. L., Saad, C. R. Effects of *Bacillus subtilis* on the growth performance, digestive enzymes, immune gene expression, and disease resistance of white shrimp *Litopenaeus vannamei*. Fish & Shellfish Immunology. 2012. 33(4), 683–689.
40. Gao, Y., Zhou, Z., Jiao, Y., Xu, Y., Li, W., & Liu, Y. Effects of dietary synbiotic supplementation on growth performance, feed utilization, body composition and intestinal morphology in juvenile GIFT tilapia (*Oreochromis niloticus*). Aquaculture Nutrition. 2013. 19(3), 371–379. https://doi.org/10.1111/j.1365-2095.2012.00975.x.
41. Chen, J. C., & Chen, C. T. Effects of ammonia and nitrite on immune response of white shrimp *Litopenaeus vannamei*. Fish & Shellfish Immunology. 2001. 11(4), 311–322.
42. Gibson, G. R., Probert, H. M., Van Loo, J., Rastall, R. A., & Roberfroid, M. B. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutrition Research Reviews. 2004. 17(2), 259–275. <https://doi.org/10.1079/NRR200479>.
43. Avnimelech, Y. (1999). Carbon/nitrogen ratio as a control element in aquaculture systems. Aquaculture, 176(3-4), 227–235. <https://doi.org/10.1016/S0044-8486> (99)00085-X.

 **The references used by the researcher are good but most of them are old references, and the research needs to add other modern references and it is preferable for the researcher to follow one method in writing scientific sources**