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Probiotics and prebiotics (MOS, FOS, XOS and Combinations): Synergistic action on the antioxidant capacity and immune parameters of *Litopenaeus vannamei*

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Abstract
The present study evaluated the immunomodulatory and antioxidative responses of *Litopenaeus vannamei* fed with diets supplemented with probiotics, prebiotics (mannan-oligosaccharides [MOS], fructo-oligosaccharides [FOS], and xylo-oligosaccharides [XOS]), and their combinations. Twelve experimental treatments were formulated, including a control (T1), individual and combined prebiotics, probiotics, and synbiotics. Key immune parameters such as Total Haemocyte Count (THC), Phagocytic Activity (PA), Melanin Production, and levels of immunoglobulin-like proteins were measured alongside antioxidant markers including Superoxide Dismutase (SOD), Catalase (CAT), and Total Antioxidant Capacity (TAC). Results revealed significant enhancements ($p < 0.05$) in all immunological and antioxidative indices across the treatment groups compared to the control, with the highest values consistently observed in the T12 group (MOS + FOS + XOS + Probiotic). This indicates a synergistic effect between the prebiotic components and probiotic strains (*Bacillus licheniformis* and *Lactobacillus rhamnosus*), leading to improved immune defense and oxidative stress resilience in shrimp. The findings suggest that synbiotic-based functional feeds can be a promising strategy for boosting shrimp health and immunity in intensive aquaculture systems. These natural feed additives offer a sustainable alternative to conventional chemotherapeutics, potentially improving disease resistance and production outcomes in commercial shrimp farming.

Keywords: Synbiotics, Probiotics, Prebiotics (MOS, FOS, XOS), *Litopenaeus vannamei*

1. Introduction
Shrimp aquaculture plays a pivotal role in global seafood production, with *Litopenaeus vannamei* (Pacific whiteleg shrimp) dominating international markets due to its favorable traits such as rapid growth, high survival rate, and tolerance to diverse salinity and temperature ranges [1]. However, the intensification of shrimp farming systems has been accompanied by serious ecological and health-related challenges, including disease outbreaks, immunosuppression, environmental stress, and poor water quality. These problems often stem from microbial imbalances, accumulation of organic matter, and oxidative stress, all of which compromise shrimp immunity and survival [2, 3].

To address these issues, researchers and industry stakeholders have shifted focus towards sustainable, non-antibiotic approaches to enhance shrimp health and reduce environmental impact. One promising strategy is the incorporation of functional feed additives such as probiotics, prebiotics, and synbiotics, which serve as eco-friendly immunostimulants and health enhancers in aquaculture [4, 5]. Probiotics are defined as live microbial supplements that beneficially affect the host by improving intestinal microbial balance [6]. In shrimp aquaculture, commonly used probiotic strains include *Bacillus spp.* and *Lactobacillus spp.*, which exert multiple beneficial effects including enhancement of innate immune responses, competitive exclusion of pathogens, secretion of antimicrobial compounds, and improvement of digestive enzyme activity [7, 8]. These microbes also help in reducing stress-induced oxidative damage by stimulating antioxidant enzyme systems such as superoxide dismutase (SOD) and catalase (CAT) [9].

Prebiotics, on the other hand, are selectively fermented non-digestible oligosaccharides that stimulate the growth or activity of beneficial gut microbiota [10]. Among the most studied prebiotics in aquaculture are mannan-oligosaccharides (MOS), fructo-oligosaccharides (FOS), and xylo-oligosaccharides (XOS). MOS has been shown to inhibit pathogenic bacteria by preventing their adhesion to gut mucosa, while FOS and XOS improve nutrient utilization and promote the growth of lactic acid bacteria in the gastrointestinal tract [11, 12]. These effects cumulatively strengthen the immune barrier, enhance antioxidant responses, and improve disease resistance in shrimp [13]. The integration of probiotics and prebiotics into a synbiotic formulation is hypothesized to exert synergistic effects by simultaneously introducing beneficial microbes and enhancing their colonization and metabolic activity through the presence of selective substrates [14]. Such synbiotic interventions are increasingly recognized for their potential to significantly improve shrimp immune response, gut morphology, antioxidant capacity, and resistance to pathogens like *Vibrio harveyi* and *White Spot Syndrome Virus (WSSV)* [15, 16]. Despite the growing interest in synbiotics, limited studies have explored the combined supplementation of multiple prebiotics (MOS, FOS, XOS) along with probiotics and their cumulative impact on immune and antioxidant responses in *L. vannamei*. Additionally, comparative data among different formulations are scarce, making it difficult to identify the most effective combinations for practical application. Therefore, the objective of this study was to investigate the synergistic effects of dietary probiotics and prebiotics, individually and in combination, on immune parameters such as total haemocyte count (THC), phagocytic activity, melanin production, and immunoglobulin levels, as well as antioxidant markers including SOD, CAT, and total antioxidant capacity (TAC) in *Litopenaeus vannamei*. This research aims to contribute to the development of optimized synbiotic feeding strategies that can enhance shrimp immunity and resilience, thereby supporting more sustainable and profitable aquaculture practices.

2. Materials and Methods

2.1. Experimental Site and Animal Husbandry

The study was carried out at shrimp culture units located in Allur, Nellore District, Andhra Pradesh, India (Latitude: 15.00120° N; Longitude: 80.05200° E). Juvenile *Litopenaeus vannamei* (Pacific white shrimp) were sourced from local aquaculture farms and transported to the experimental facility in oxygen-filled, double-layered polythene bags to minimize transport-related stress. Shrimp of uniform size were selected after comprehensive disease screening to ensure a pathogen-free stock. The selected shrimp were acclimatized in tanks containing seawater adjusted to the experimental salinity. The acclimation period lasted one week under field conditions, during which the shrimp were fed twice daily with a commercial feed containing 35% crude protein (CP).

2.2. Experimental Setup

The feeding trial was conducted in cement tanks (5 × 10 m) with earthen bottoms embedded in the submerged earth crust. Each tank maintained a water depth of 1 meter and held a total volume of 3000 liters. Seawater with a salinity of 10 ± 0.5 ppt was used throughout the experiment. Water was sourced from the Buckingham Canal, treated in a sedimentation pond, and then distributed to all experimental units. Continuous aeration was provided via electric blowers and air compressors to maintain optimal dissolved oxygen levels. Tanks were covered with black netlon plastic sheets to regulate photoperiod and temperature, maintaining a 12 h light: 12 h dark natural cycle. Water exchange was performed twice weekly, and dechlorinated seawater was added regularly to compensate for evaporation.

2.3. Experimental Design and Feeding Protocol

The experiment aimed to evaluate the individual and synergistic effects of probiotics and prebiotics on the growth performance of *Litopenaeus vannamei*. Twelve dietary treatments were formulated using different combinations of prebiotics—mannan-oligosaccharides (MOS), fructo-oligosaccharides (FOS), and xylo-oligosaccharides (XOS)—and probiotics, including *Lactobacillus rhamnosus* and *Bacillus licheniformis*. Treatments included both single and combined applications of these components.

A completely randomized design (CRD) was employed, with the dietary treatments considered as independent variables. Acclimatized shrimp with an initial average body weight of 0.88 ± 0.06 g were randomly stocked in the experimental tanks. Shrimp were initially fed at 10% of their body weight, with the feeding rate gradually reduced to 3-4% towards the end of the 60-day trial. The daily feed allocation was adjusted weekly based on the average body weight obtained through subsampling. Feed was administered in two equal portions daily at 06:00 and 18:00 hours.

The 12 treatments were as follows:

1. **Control Diet (T1):** Basal diet without any supplementation.
2. **Probiotic Diet (T2):** Basal diet supplemented with the probiotic *Lactobacillus plantarum*.
3. **MOS Diet (T3):** Basal diet supplemented with Mannan-oligosaccharides (MOS).
4. **FOS Diet (T4):** Basal diet supplemented with Fructo-oligosaccharides (FOS).
5. **XOS Diet (T5):** Basal diet supplemented with Xylo-oligosaccharides (XOS).
6. **MOS + Probiotic Diet (T6):** Basal diet supplemented with MOS and *Lactobacillus plantarum*.
7. **FOS + Probiotic Diet (T7):** Basal diet supplemented with FOS and ***Lactobacillus rhamnosus* and *Bacillus licheniformis***.
8. **XOS + Probiotic Diet (T8):** Basal diet supplemented with XOS and ***Lactobacillus rhamnosus* and *Bacillus licheniformis***.
9. **MOS + FOS + Probiotic Diet (T9):** Basal diet supplemented with MOS, FOS, and ***Lactobacillus rhamnosus* and *Bacillus licheniformis***.
10. **MOS + XOS + Probiotic Diet (T10)**: Basal diet supplemented with MOS, XOS, and ***Lactobacillus rhamnosus* and *Bacillus licheniformis***.
11. **FOS + XOS + Probiotic Diet (T11):** Basal diet supplemented with FOS, XOS, and ***Lactobacillus rhamnosus* and *Bacillus licheniformis***.
12. **MOS + FOS + XOS + Probiotic Diet (T12):** Basal diet supplemented with MOS, FOS, XOS, and ***Lactobacillus rhamnosus* and *Bacillus licheniformis***.

2.4. Preparation of Probiotic Feed and Supplementation

Probiotic-enriched feed was prepared following the methodologies described by Naresh [17] and Aparna [18]. The bacterial strains *Lactobacillus rhamnosus* and *Bacillus* *licheniformis* were cultured in nutrient broth and harvested by centrifugation at 10,000 rpm for 10 minutes. The cell pellets were washed with phosphate-buffered saline (PBS, pH 7.4) and re-suspended in the same buffer. The bacterial suspension was then uniformly sprayed onto the basal feed pellets to ensure even distribution. The coated pellets were subsequently dried at 40 °C, sealed in airtight polythene bags, and stored under refrigeration until use.

The final probiotic feed contained *B. licheniformis* and *L. rhamnosus* at a concentration of 10^{10} CFU/kg and was freshly prepared on a weekly basis.

The basal diet was formulated to meet the nutritional requirements of *Litopenaeus vannamei*, with an approximate composition of 35% protein, 7% lipid, 15% carbohydrate, and 10% fiber. Key ingredients included fishmeal, soybean meal, wheat flour, and a vitamin-mineral premix, providing a nutritionally balanced formulation for optimal shrimp growth [18].

2.5. Prebiotic Supplementation

Three commercially available prebiotics—mannan-oligosaccharides (MOS), fructo-oligosaccharides (FOS), and xylo-oligosaccharides (XOS)—were incorporated into the feed at concentrations of 2%, 1.5%, and 1%, respectively. The prebiotics were thoroughly mixed with the basal feed to ensure uniform distribution throughout the diet.

Hemolymph from shrimp was collected from the ventral sinus using sterile syringe preloaded with anticoagulant. Hemolymph was mixed thoroughly and kept in ice to prevent clotting and melanisation. After centrifugation hemocyte pellet was re suspended in cold phosphate buffer solution and subsequently used for experimental purposes. Total hemocyte count (THC) was estimated using Neubauer Hemocytometer under Light Microscope (at 400 X magnification) after dilution of shrimp hemolymph in an anticoagulant to prevent clotting. Counting all cells in the four large corner squares of the grid (each square = 1mm^2). Phagocyte activity (%) was calculated by following formula [19, 20].

$$\frac{\text{Number of Phagocyte hemocytes}}{\text{Total number of hemocytes counted}} \text{ X } 100$$

Melanin production rate in shrimp hemocytes was typically estimated by the formation of dopachrome from L-DOPA, by adopting the Specttrophotometric method[20]. Immunoglobulins or Immune related proteins was estimated by adopting the procedure of Liu [21]. Total Antioxidant capacity (TAC) was measured by following the method Benzie & Stain (Ref). Superoxide dismutase (SOD) and Catalase (CAT) were estimated the procedures outlined by Kakkar *et al.* [22] and Takahara *et al.* [23], respectively.

2.7. Statistical Analysis

A two-way Analysis of Variance (ANOVA) was employed to evaluate the effects of probiotic and prebiotic supplementation—individually and in combination—on the immune parameters and Antioxidant capacity of *Litopenaeus vannamei*. The analysis considered treatment and time as independent variables. Significant differences among treatment groups were determined at a confidence level of $p < 0.05$. Where significant differences were detected, Tukey's Honest Significant Difference (HSD) post-hoc test was applied to identify pairwise differences between treatments. Results indicated that Treatment T12 (combination of MOS + FOS + XOS + Probiotic) yielded the highest rates, significantly outperforming all other treatments, including the control group (T1).

3. Results and Discussion

The immunological and antioxidant responses of *Litopenaeus vannamei* varied significantly among the different dietary treatments. The tested parameters included Total Haemocyte Count (THC), Phagocytic Activity, Melanin Production, Superoxide Dismutase (SOD) Activity, Catalase Activity, Total Antioxidant Capacity (TAC), and Immunoglobulin levels. The data showed progressive enhancement across treatments from T1 (Control) to T12 (combined supplementation of MOS + FOS + XOS + Probiotic). Results are presented below.

3.1. Total Haemocyte Count (THC)

The THC is a critical indicator of shrimp immune status. The control group (T1) exhibited the lowest THC ($3.8 \pm 0.2 \times 10^6$ cells/ml), while treatment groups supplemented with probiotics and/or prebiotics showed marked increases. T6 (Probiotic only) showed a THC of $5.1 \pm 0.4 \times 10^6$ cells/ml, indicating a 34% rise over control. The highest THC value was observed in T12 ($5.9 \pm 0.4 \times 10^6$ cells/ml), accounting for a 55% increase. Other high-performing groups included T11 (5.7 ± 0.4), T10 (5.4 ± 0.3), and T9 (5.5 ± 0.3). The trend clearly shows that groups receiving combinations of multiple oligosaccharides with probiotics (T9-T12) had significantly ($p < 0.05$) higher THC than single supplementation groups.

3.2. Phagocytic Activity (PA%)

Phagocytic activity steadily increased from $38 \pm 2\%$ in the control group to $58 \pm 3\%$ in T12, indicating enhanced cellular immunity. Moderate increases were seen in T2 to T5 (42-46%), with significant enhancements observed from T6 (50%) onward. The synbiotic treatments (T9-T12) consistently recorded higher phagocytic activity (55-58%), with T12 showing the peak response (+53% over control). These results demonstrate that the synergistic effects of prebiotics and probiotics significantly stimulated haemocyte-mediated immunity.

3.3. Melanin Production

Melanin production, indicative of prophenoloxidase system activity, ranged from 0.27 ± 0.04 mg/g in T1 to 0.42 ± 0.04 mg/g in T12. Notable increases were observed in treatments with synbiotic supplements. T6 (probiotic) showed an intermediate value of 0.36 ± 0.05 mg/g, while T9-T12 showed superior values (0.38-0.42 mg/g). The increase in melanin production was statistically significant ($p < 0.05$) for all groups beyond T3. The highest percent difference change (+56%) was found in T12, reaffirming the enhanced immune response.

3.4. Superoxide Dismutase (SOD) Activity

SOD activity, a key antioxidant enzyme that neutralizes superoxide radicals, improved across all treatments compared to the control (T1: 1.13 ± 0.11 u/mg). T6 (probiotic only) showed an activity of 1.53 ± 0.14 u/mg, while the synbiotic groups (T9 to T12) recorded values between 1.74 and 2.08 u/mg. The highest SOD activity was observed in T12 (2.08 ± 0.14 u/mg), marking an 84% improvement over the control. These differences were statistically significant

($p < 0.05$), particularly in groups receiving multi-oligosaccharides in combination with probiotics.

3.5. Catalase Activity
Catalase activity, which aids in the breakdown of hydrogen peroxide, also showed upward trends across treatments. T1 showed the lowest catalase value (0.23 ± 0.02 u/mg), whereas T12 recorded the highest value (0.42 ± 0.03 u/mg), denoting an 82% enhancement. Significant improvements were seen in T6 to T12, especially in groups receiving multiple prebiotics and probiotics. The treatments T9-T12 (combinations) had consistently higher values (0.34-0.42 u/mg) than single supplement treatments, suggesting improved oxidative stress defense.

3.6. Total Antioxidant Capacity (TAC)
TAC values followed a similar increasing trend. T1 recorded the lowest TAC (1.58 ± 0.14), while T12 recorded the highest (2.83 ± 0.22), translating to a +79% increase. Treatments T6-T12 significantly outperformed the control, with T9-T12 showing statistically significant ($p < 0.05$) increases over single-component treatments. These results indicate that combined supplementation promoted a more efficient and cumulative antioxidant response, vital for cellular protection under stressful aquaculture conditions.

3.7. Immunoglobulin Levels
Serum immunoglobulin levels increased consistently with the complexity of supplementation. T1 had the lowest level (0.31 ± 0.04 mg/ml), while T12 showed the highest (0.61 ± 0.04 mg/ml), reflecting a +97% difference. Early treatments (T2-T4) showed modest increases (0.37-0.44 mg/ml), while substantial rises were evident from T6 onwards. Treatments T9 through T12 showed highly significant increases in immunoglobulin concentration ($p < 0.05$), confirming the role of synbiotic diets in enhancing humoral immunity.

4. Discussion
The present study demonstrated that dietary supplementation with probiotics, prebiotics, and their synbiotic combinations significantly enhanced both immune and antioxidant responses in *Litopenaeus vannamei*. These findings align with and expand upon existing literature, underscoring the efficacy of functional feed additives in shrimp aquaculture.

4.1 Immune Responses
A clear upward trend in Total Haemocyte Count (THC), Phagocytic Activity, and Melanin Production was observed across the treated groups, particularly in T9-T12, which received combinations of MOS, FOS, XOS, and probiotics. THC is an essential non-specific immune marker in crustaceans and directly relates to disease resistance capacity. The significantly elevated THC in synbiotic-treated groups corroborates previous findings by Zokaeifar *et al.* [24], who reported enhanced THC in *L. vannamei* fed a synbiotic diet comprising *Bacillus* spp. and inulin.

Phagocytic activity, representing haemocyte function, also increased significantly. The enhancement in PA% suggests activation of cellular immune mechanisms, likely due to the immunostimulatory role of probiotics such as *Lactobacillus rhamnosus* and *Bacillus licheniformis*, and prebiotics which selectively stimulate beneficial gut microbes. Similar immunopotentiating effects were observed in studies by Hai *et al.* [25] and Dawood and Koshio [26], where probiotics enhanced phagocytosis and respiratory burst in shrimp.

Melanin synthesis, a downstream response of the prophenoloxidase (proPO) cascade, was notably higher in synbiotic groups. This pathway plays a crucial role in pathogen encapsulation and wound healing. Wang *et al.* [27] highlighted the importance of increased proPO activity and melanin deposition in shrimp fed with combined dietary immunostimulants.

4.2 Antioxidant Responses
Oxidative stress is a major challenge in intensive shrimp aquaculture, often resulting from crowding, water quality fluctuations, and pathogen exposure. The increased SOD and Catalase activity observed in this study indicate an enhanced antioxidative defense system, crucial for maintaining cellular homeostasis. These enzymes catalyze the dismutation of superoxide radicals and hydrogen peroxide, respectively, thereby minimizing oxidative damage. The current results are consistent with Moriarty [28] and Yuan *et al.* [29], who reported increased antioxidant enzyme activities in shrimp following probiotic administration.

The Total Antioxidant Capacity (TAC) values, which reflect cumulative antioxidant status, were also significantly higher in synbiotic-fed groups. This suggests that the combination of probiotics and prebiotics induces synergistic effects that enhance both enzymatic and non-enzymatic antioxidant systems. Shao *et al.* [30] and Xie *et al.* [31] demonstrated similar findings in *Penaeus monodon* and *L. vannamei*, attributing increased TAC to enhanced gut microbial balance and nutrient bioavailability.

4.3 Humoral Immunity
The marked elevation in serum immunoglobulin levels in T9-T12 indicates a robust activation of the humoral immune response. Although crustaceans lack true immunoglobulins like vertebrates, immunoglobulin-like proteins or pattern recognition proteins (PRPs) function similarly in pathogen recognition and opsonization. Synbiotic diets may upregulate these molecules via gut-associated immune modulation. This observation is supported by Wang *et al.* [32], who noted increased expression of PRPs and antimicrobial peptides in shrimp fed synbiotic-supplemented diets.

4.4 Synergistic Effects of Synbiotics
Notably, the combination of multiple oligosaccharides (MOS + FOS + XOS) with probiotics in T_{12} yielded the most substantial improvements across all parameters. The synergistic effects are likely due to:

1. **Enhanced microbial colonization and stability** in the gut by prebiotics that serve as substrates for beneficial bacteria;
2. **Improved nutrient absorption and immune signaling**, facilitated by probiotics;
3. **Reduced oxidative damage** due to better enzymatic antioxidant defenses.

These findings confirm the synergistic action of synbiotics, as proposed by Guerreiro *et al.* [33] and Panigrahi *et al.* [34], who highlighted that combinations of functional feed additives provide multifaceted benefits compared to individual supplementation.

4.5 Synergistic Effects of Combined Prebiotics and Probiotics

The superior performance of synbiotic groups (Especially T_{12}) can be attributed to the synergistic interaction between prebiotics and probiotics. Prebiotics such as MOS, FOS, and XOS act as fermentable substrates that selectively enhance the growth of administered probiotics as well as native beneficial gut bacteria (e.g., *Lactobacillus*, *Bifidobacterium*). This improved microbial balance enhances intestinal morphology, digestive enzyme production, and nutrient absorption, all of which contribute to improved immune competence.

Moreover, each oligosaccharide may exert distinct and complementary effects. For instance:

- MOS binds pathogenic bacteria (like *Vibrio spp.*), preventing their adhesion to the gut epithelium (Spring *et al*., 2000)[35].
- FOS and XOS promote the proliferation of lactic acid bacteria, improving gut pH and barrier function.

The presence of all three oligosaccharides may thus create a broad-spectrum prebiotic effect, ensuring optimal support for the probiotic strains used. This concept is supported by Wang *et al*. [36] and Geng *et al*. [37], who documented enhanced gut immunity and resistance to white spot syndrome virus (WSSV) in *L. vannamei* under synbiotic feeding.

4.6 Implications for Shrimp Health and Aquaculture

From a practical aquaculture perspective, these immunological and antioxidative enhancements suggest that synbiotic feeding strategies can:

- Improve shrimp resistance to opportunistic pathogens;
- Reduce dependence on antibiotics and chemical immunostimulants;
- Enhance overall shrimp survival, health, and potentially growth.

This is especially relevant in the context of sustainable aquaculture, where functional feeds that enhance disease resistance and promote health without environmental side effects are in high demand. The integration of synbiotics into feed formulation could serve as a preventive health management strategy in commercial shrimp hatcheries and grow-out systems.

Table 1: Anti oxidant capacity and Immune parameters of *L.vannamei* subjected different Experimental feeding trails

	Treatments											
Parameter	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
Total Haemocyte Count (THC)	3.8±0.2	4.4±0.3	4.7±0.3	4.8±0.3	4.5±0.3	5.1±0.4	5.3±0.3	5.0±0.3	5.5±0.3	5.4±0.3	5.7±0.4	5.9±0.4
(x 10^6 cells/ml)	PDC	$(+16)^a$	$(+24)^b$	$(+26)^b$	$(+18)^a$	$(+34)^b$	$(+40)^{b,c}$	$(+32)^b$	$(+45)^{b,c}$	$(+42)^{b,c}$	$(+50)^{b,c}$	$(+55)^{b,c}$
Phagocytic Activity (%)	38	42	44	46	45	50	52	51	55	54	56	58
	±2	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3
	PDC	$(+11)^a$	$(+16)^a$	$(+21)^b$	$(+19)^a$	$(+32)^{b,c}$	$(+37)^{b,c}$	$(+34)^{b,c}$	$(+45)^{b,c}$	$(+42)^{b,c}$	$(+48)^{b,c,d}$	$(+53)^{b,c,d}$
Melanin Production (mg/g)	0.27	0.30	0.31	0.33	0.32	0.36	0.37	0.35	0.38	0.39	0.40	0.42
	±0.04	±0.05	±0.06	±0.05	±0.05	±0.05	±0.05	±0.04	±0.04	±0.05	±0.04	±0.04
	PDC	$(+11)^a$	$(+15)^a$	$(+22)^b$	$(+19)^a$	$(+33)^{b,c}$	$(+37)^{b,c}$	$(+30)^{b,c}$	$(+41)^{b,c,d}$	$(+45)^{b,c,d}$	$(+48)^{b,c,d}$	$(+56)^{b,c,d}$
SOD Activity (u/mg protein)	1.13	1.25	1.32	1.41	1.37	1.53	1.63	1.54	1.74	1.73	1.84	2.08
	±0.11	±0.12	±0.12	±0.13	±0.12	±0.14	±0.13	±0.14	±0.13	±0.12	±0.13	±0.14
	PDC	$(+11)^a$	$(+17)^a$	$(+25)^b$	$(+21)^b$	$(+36)^{b,c}$	$(+44)^{b,c}$	$(+36)^{b,c}$	$(+54)^{b,c,d}$	$(+53)^{b,c,d}$	$(+63)^{b,c,d}$	$(+84)^{b,c,d}$
Catalase (u/mg protein)	0.23	0.27	0.29	0.28	0.27	0.31	0.33	0.32	0.34	0.35	0.38	0.42
	±0.02	±0.03	±0.03	±0.03	±0.03	±0.3	±0.03	±0.03	±0.03	±0.03	±0.03	±0.03
	PDC	$(+18)^a$	$(+26)^a$	$(+22)^a$	$(+17)^a$	$(+35)^{b,c}$	$(+44)^{b,c}$	$(+39)^{b,c,d}$	$(+48)^{b,c,d}$	$(+52)^{b,c,d}$	$(+65)^{b,c,d}$	$(+82)^{b,c,d}$
Total Antioxidant Capacity (TAC)	1.58	1.83	1.92	2.11	2.23	2.34	2.45	2.57	2.59	2.64	2.74	2.83
	±0.14	±0.16	±0.17	±0.16	±0.16	±0.17	±0.18	±0.18	±0.19	±0.19	±0.21	±0.22
	PDC	$(+16)^a$	$(+22)^a$	$(+34)^b$	$(+41)^b$	$(+48)^{b,c}$	$(+55)^{b,c}$	$(+62)^{b,c}$	$(+64)^{b,c,d}$	$(+67)^{b,c,d}$	$(+73)^{b,c,d}$	$(+79)^{b,c,d}$
Immunoglobulin (mg/ml)	0.31	0.37	0.4	0.44	0.46	0.48	0.49	0.52	0.55	0.57	0.59	0.61
	±0.04	±0.04	±0.04	±0.04	±0.04	±0.03	±0.04	±0.04	±0.03	±0.04	±0.04	±0.04
	PDC	$(+19)^a$	$(+29)^b$	$(+42)^{b,c}$	$(+48)^{b,c}$	$(+55)^{b,c}$	$(+58)^{b,c}$	$(+67)^{b,c,d}$	$(+77)^{b,c,d}$	$(+83)^{b,c,d}$	$(+90)^{b,c,d}$	$(+97)^{b,c,d}$

All values are Mean± SD of Six Individual Observations

PDC: Per cent Deviation over Respective Control

Values with different Superscripts are significantly different from each other @ $p< 0.05$

5. Conclusion

The present investigation conclusively demonstrates that the dietary incorporation of probiotics, prebiotics (MOS, FOS, and XOS), and their combinations (synbiotics) significantly enhances the immunological and antioxidant defense mechanisms of *Litopenaeus vannamei*. Among the twelve experimental treatments, the combination treatment (T12: MOS + FOS + XOS + Probiotic) consistently exhibited the highest levels of total haemocyte count, phagocytic activity, melanin production, enzymatic antioxidants (SOD, Catalase), total antioxidant capacity, and immunoglobulin-like proteins. This indicates a robust synergistic effect between selected probiotics and prebiotics, leading to a more comprehensive immune response and improved stress tolerance in cultured shrimp.

These findings provide strong evidence that:

- Probiotics enhance immune functions by modulating gut microbiota, improving intestinal integrity, and stimulating cellular and humoral immune responses.
- Prebiotics selectively nourish beneficial bacteria and facilitate metabolite production (e.g., SCFAs) that directly modulate host immunity.
- Synbiotic feeding results in additive or synergistic benefits, optimizing both microbial balance and host physiological resilience.

Such functional feed interventions not only reduce the risk of disease outbreaks but also offer a sustainable alternative to chemotherapeutics and antibiotics, aligning with modern aquaculture practices that emphasize eco-friendliness, biosafety, and long-term productivity. Moreover, the enhancement of antioxidant enzyme systems suggests that synbiotics help mitigate oxidative stress, a common consequence of environmental fluctuations and pathogenic invasions in intensive shrimp farming. This contributes to improved survival, health, and potentially growth performance under commercial conditions.

In summary, this study substantiates the strategic value of synbiotic dietary formulations in enhancing shrimp immunity and oxidative stress resilience. It offers a promising, holistic approach to fortifying shrimp health and sustainability in the face of growing challenges in modern aquaculture.

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