E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 76.37
(GIF) Impact Factor: 0.549
IJFAS 2025; 13(3): 297-305

www.fisheriesjournal.com
Received: 12-03-2025
Accepted: 15-04-2025

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Synergistic action of probiotics and prebiotics (Mos, Fos, XOS and combinations) on the growth performance of *Litopenaeus vannamei*

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DOI: https://www.doi.org/10.22271/fish.2025.v13.i3d.3101

Abstract
The shrimp farming industry has been facing challenges with maintaining optimal growth and health of *Litopenaeus vannamei* due to poor water quality, pathogens, and feed-related issues. Probiotics and prebiotics, particularly mannose oligosaccharides (MOS), fructose oligosaccharides (FOS), and xylose oligosaccharides (XOS), have garnered attention for their potential to enhance gut health, promote growth, and strengthen immunity in aquatic organisms. This study aimed to assess the synergistic effects of probiotics and prebiotics (MOS, FOS, XOS) and their combinations on the growth patterns of *L. vannamei*. Twelve dietary treatments were administered to shrimp over a 60 day experimental feeding trail period, and growth parameters, such as weight gain, survival rate, feed conversion ratio (FCR), and specific growth rate (SGR), were recorded. T_1 (Control), T_2 (Probiotic only), T_3 (MOS only), T_4 (FOS only), T_5 (XOS only), T_6 (MOS + Probiotic), T_7 (FOS + Probiotic), T_8 (XOS + Probiotic), T_9 (MOS + FOS + Probiotic), T_{10} (MOS + XOS + Probiotic), T_{11} (FOS + XOS + Probiotic), and T_{12} (MOS + FOS + XOS + Probiotic) were the twelve dietary treatments that were conducted. The results indicate that probiotic and prebiotic supplementation, particularly in combination, significantly enhanced growth and health indices compared to the control group. Among the combinations, the MOS + FOS + Probiotic treatment (T_{12}) showed the most promising results, yielding a significant improvement in growth rates and feed efficiency. These findings highlight the promising role of synbiotics in improving shrimp aquaculture efficiency.

Keywords: Probiotics, prebiotics, synbiotics, *Litopenaeus vannamei*, growth performance, Mannan-oligosaccharides (MOS), Fructo-oligosaccharides (FOS), XYLO-oligosaccharides (XOS)

1. Introduction
Aquaculture, as one of the fastest-growing sectors in global food production, faces significant challenges related to the health, growth, and sustainability of farmed species. Among the many species cultured in aquaculture, *Litopenaeus vannamei* holds great commercial importance due to its rapid growth rate, adaptability to varying environmental conditions, and high market demand. However, the shrimp farming industry is confronted with multiple issues, including disease outbreaks, poor feed efficiency, and environmental stress, all of which impact the overall productivity and profitability of shrimp farming operations [1, 2]. Traditional approaches to improving shrimp health, such as antibiotics and chemicals, have been met with growing concerns due to their negative environmental impacts, the emergence of antibiotic-resistant pathogens, and the increasing demand for organic and sustainable farming practices [3, 4]. As a result, alternative approaches that enhance shrimp health, promote growth, and improve feed utilization are being actively explored.

One promising approach involves the use of probiotics and prebiotics in aquaculture diets. Probiotics, defined as live microorganisms that confer health benefits to the host when administered in adequate amounts, are known to improve gut health, immune function, and disease resistance in aquatic organisms [5, 6]. Probiotics help to balance the intestinal microbiota, suppress harmful pathogens, enhance digestion and nutrient absorption, and boost immune responses [5, 7].

In shrimp farming, probiotics have been shown to improve growth performance, disease resistance, and feed conversion efficiency [8, 9]. However, the effects of probiotics can be further enhanced when combined with prebiotics.

Prebiotics are non-digestible food ingredients, such as oligosaccharides, that selectively stimulate the growth and/or activity of beneficial microorganisms in the gut. Prebiotics have been used to enhance the growth of probiotics and other beneficial bacteria, further improving gut health and the overall well-being of the host organism [7, 8, 10]. In aquaculture, various types of prebiotics have been investigated, including mannose oligosaccharides (MOS), fructose oligosaccharides (FOS), and xylose oligosaccharides (XOS). MOS, derived from the cell wall of *Saccharomyces cerevisiae*, is known to bind to pathogenic bacteria, preventing them from attaching to the intestinal lining of shrimp and other aquatic organisms. FOS and XOS are carbohydrate polymers that serve as substrates for beneficial gut microbes, promoting their proliferation and activity. These prebiotics have been found to improve feed utilization, enhance immune function, and reduce the growth of harmful pathogens in several aquaculture species [11, 12].

Although probiotics and prebiotics have individually demonstrated benefits for shrimp growth and health, limited research has explored the synergistic effects of combining these supplements, particularly when different prebiotics are used together. The potential for synergistic effects arises from the complementary mechanisms by which these dietary components interact with the gut microbiota, modulating its composition and activity in ways that optimize nutrient absorption, immune responses, and overall health. In particular, combinations of probiotics with prebiotics, such as MOS, FOS, and XOS, may produce enhanced benefits over their individual use, thus improving the sustainability and efficiency of shrimp farming operations [8, 9, 11].

This study aims to investigate the effects of probiotics and different prebiotics, individually and in combination, on the growth performance of *Litopenaeus vannamei* under controlled aquaculture conditions. Specifically, the study will evaluate the effects of mannose oligosaccharides (MOS), fructose oligosaccharides (FOS), and xylose oligosaccharides (XOS) when added to a basal shrimp diet, either as individual supplements or in various combinations, alongside a probiotic strains *Lactobacillus rhamnosus* and *Bacillus licheniformis*. This research will assess key growth parameters, such as weight gain, specific growth rate (SGR), feed conversion ratio (FCR), and survival rate, to determine the most effective combinations of these dietary components. Additionally, the study will provide insights into how these prebiotic-probiotic interactions may contribute to enhanced feed utilization, disease resistance, and overall shrimp health. This study aims to evaluate the individual and combined effects of these prebiotics along with probiotics on the growth performance of *L. vannamei*.

2. Materials and Methods

2.1. Experimental Site and Animal Husbandry

The present study was conducted at shrimp culture units located in Allur (Latitude 15.00120° N; Longitude 80.05200° E), Nellore District, Andhra Pradesh, India. Specimens of *Litopenaeus vannamei* (Penaeid shrimp) were procured from local aquaculture ponds and transported to the experimental facility in oxygenated, double-layered polythene bags to minimize handling stress. Shrimp of uniform size were selected following thorough disease screening to ensure a pathogen-free stock. The selected individuals were transferred to acclimatization tanks containing seawater adjusted to the desired salinity. A one-week acclimatization period was maintained under field conditions, during which the shrimp were fed twice daily with a commercial formulated diet containing 35% crude protein (CP).

2.2. Experimental Setup

The feeding trial was conducted in cement tanks (5 × 10 m) embedded in submerged earth crust with earthen bottoms. Each tank had a water depth maintained at 1 meter, with a total water volume capacity of 3000 liters. Seawater with a salinity of 10 ± 0.5 ppt was used, and water for the experiment was drawn from the Buckingham Canal, treated in a designated pond, and subsequently distributed to all experimental tanks. Aeration was continuously provided using electric blowers connected to air compressors to ensure optimal dissolved oxygen levels. All tanks were covered with black netlon plastic sheets to regulate photoperiod and temperature, and shrimp were maintained under a natural 12 h light: 12 h dark cycle. Water exchange was carried out twice weekly, and dechlorinated seawater was added periodically to compensate for evaporation losses.

2.3. Experimental Design and Feeding Protocol

This experiment was conducted to evaluate the individual and combined effects of probiotics and prebiotics on the growth performance of *Litopenaeus vannamei*. The study involved 12 different dietary treatments, each incorporating different combinations of prebiotics (Mannan-oligosaccharides (MOS), Fructo-oligosaccharides (FOS), Xylo-oligosaccharides (XOS)) and probiotics including *Lactobacillus rhamnosus* and *Bacillus licheniformis*. The experimental treatments included both single and combined supplementation of these components.

The study was arranged using a completely randomized design (CRD) involving various experimental treatments as independent variables. Acclimatized shrimp of uniform body weight (0.88 ± 0.06 g) were randomly stocked in the experimental tanks. Shrimp were initially fed experimental diets at 10% of their body weight, with the feeding rate gradually adjusted to 3-4% by the end of the 60-day feeding trial experimental period. The daily ration for each treatment group was calculated based on the weekly average body mass of shrimp obtained from subsampling. Feed was distributed in two equal portions daily at 06:00 and 18:00 hours.

The 12 treatments were as follows

1. **Control Diet (T_1):** Basal diet without any supplementation.
2. **Probiotic Diet (T_2):** Basal diet supplemented with the probiotic *Lactobacillus plantarum*.
3. **MOS Diet (T_3):** Basal diet supplemented with Mannan-oligosaccharides (MOS).
4. **FOS Diet (T_4):** Basal diet supplemented with Fructo-oligosaccharides (FOS).
5. **XOS Diet (T_5):** Basal diet supplemented with Xylo-oligosaccharides (XOS).
6. **MOS + Probiotic Diet (T_6):** Basal diet supplemented with MOS and *Lactobacillus plantarum*.
7. **FOS + Probiotic Diet (T_7):** Basal diet supplemented with FOS and *Lactobacillus rhamnosus* and *Bacillus licheniformis*.

8. **XOS + Probiotic Diet (T_8):** Basal diet supplemented with XOS and *Lactobacillus rhamnosus* and *Bacillus licheniformis.*
9. **MOS + FOS + Probiotic Diet (T_9):** Basal diet supplemented with MOS, FOS, and *Lactobacillus rhamnosus* and *Bacillus licheniformis*.
10. **MOS + XOS + Probiotic Diet (T_{10}):** Basal diet supplemented with MOS, XOS, and *Lactobacillus rhamnosus* and *Bacillus licheniformis*.
11. **FOS + XOS + Probiotic Diet (T_{11}):** Basal diet supplemented with FOS, XOS, and *Lactobacillus rhamnosus* and *Bacillus licheniformis*.
12. **MOS + FOS + XOS + Probiotic Diet (T_{12}):** Basal diet supplemented with MOS, FOS, XOS, and *Lactobacillus rhamnosus* and *Bacillus licheniformis*.

2.4. Preparation of Probiotic Feed and Supplementation:
Probiotic-enriched Feeds have been prepared following the method outlined by Naresh [13] and Aparna [14]. Bacterial species *Lactobacillus rhamnosus* as well as *Bacillus licheniformis* have been cultured in nutritional broth, extracted by centrifugation at the speed of 10,000 rpm for the time of 10 mins, washed with phosphate buffer, and re-suspended in PBS (pH 7.4). The re-suspended bacteria have been evenly mixed with the meal pellets using a spraying approach. The Probiotic mixed feed has been dried at the temp of 40 °C, then sealed in airtight polythene coverings, and kept in a refrigerator for future usage. The Probiotic feed containing *B. licheniformis* and *L. rhamnosus* at a concentration of 10 billion cfu/kg was made weekly.
The basal diet was formulated to meet the nutritional requirements of *L. vannamei*, with the following approximate composition: 35% protein, 7% lipids, 15% carbohydrates, and 10% fiber. The ingredients included fishmeal, soybean meal, wheat flour, and vitamin-mineral premix. The feed was formulated to provide a balanced nutritional profile for optimal shrimp growth (Table.1).

2.5. Prebiotic Supplementation:
Three different prebiotics were used in this study. The commercially available prebiotics including Mannan-oligosaccharides (MOS), Fructo-oligosaccharides (FOS), and Xylo-oligosaccharides (XOS) were mixed directly into the feed @ 2%, 1. & and 1% respectively, ensuring uniform distribution of each component across the diet.

2.6. Growth Parameters
Growth parameters such as growth rates, average body weights, protein efficiency ratio, feed conversion ratio, productivity rates, and feed efficiency ratio were observed and recorded. The above parameters have been determined using the following "formulas.

$$\text{Growth Rate (g/day)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Number of days}}$$

$$\text{Weight Gain (g)} = \underset{\text{At the end of the Experiment}}{\text{Weight of the Shrimp (g)}} - \underset{\text{at the start of the Experiment}}{\text{Weight of the Shrimp (g)}}$$

$$\text{Daily Growth Rates (\%)} = \frac{\text{[Initial weight X (Final weight - Initial weight)]}}{\text{Number of days of Experiment}} \text{ X 100}$$

$$\text{Daily Growth Index} = \frac{\text{Final weight}^{1/3} - \text{Initial weight}^{1/3}}{\text{Number of days of Experiment}} \text{ X 100}$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Total amount of Feed consumed (Kgs)}}{\text{Total Biomass of Shrimp (Kgs)}}$$

$$\text{Average Daily Growth Rates (ADGR)} = \frac{\underset{\text{At the end of the Expt.}}{\text{Weight of the Shrimp (g)}} - \underset{\text{at the start of the Expt.}}{\text{Weight of the Shrimp (g)}}}{\text{Total number of days of Experiment}}$$

$$\text{Specific Growth Rates (SGR)} = \frac{\underset{\text{At the end of the Experiment}}{\text{[Log weight of the shrimp (g)}} - \underset{\text{at the start of the Experiment}}{\text{Log weight of the shrimp (g)]}}}{\text{Total number of days of experiment}} \text{ x 100}$$

$$\frac{(\text{Log } W_2 - \text{Log } W_1)}{T}$$

Where:
W_1: Shrimp Weight at the start of the Experiment
W_2: Shrimp Weight at the end of the Experiment
T: Total number of days of Experiment".

Feed Efficiency Ratio (FER) = 1/Feed Conversion Ratio

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Net weight gain in wet weight (g)}}{\text{Protein consumed (g)}}$$

Survival Percentage (%)
Survival" % was determined by counting the number of shrimp in each tank after draining them at the experiment end. The survival rate percentage has been determined by utilizing the above-mentioned formula:

$$\text{Survival (\%)}: \frac{\text{Total number of animals harvested}}{\text{Total number of animals stocked}} \text{ X 100}$$

2.7 Statistical Analysis
A two-way ANOVA was performed to assess the effect of probiotics and prebiotic supplementation on growth parameters. Significant differences ($p< 0.05$) were observed between treatments. Post-hoc analysis (Tukey's HSD) revealed that T_{12} (MOS + FOS + XOS + Probiotic) had the highest growth rates and the most favorable FCR and survival rate, significantly outperforming all other treatments, including the control group (T_1).

3. Results and Discussion
In the present investigation, an attempt was made to evaluate the individual and combined effects of probiotics and prebiotics on the growth performance of *Litopenaeus vannamei*. The study comprised 12 different dietary treatments, each incorporating distinct combinations of prebiotics-namely mannan-oligosaccharides (MOS), fructo-oligosaccharides (FOS), and xylo-oligosaccharides (XOS)-along with probiotics such as *Lactobacillus rhamnosus* and *Bacillus licheniformis*. The experimental design included both

singular and combined supplementation of these functional feed additives.

Growth Performance

The growth performance of *Litopenaeus vannamei* was evaluated based on weight gain, specific growth rate (SGR), feed conversion ratio (FCR), and survival rate. A significant variation was observed among the different dietary treatments, indicating the impact of probiotic and prebiotic supplementation on the shrimp's growth and overall performance.

Weight Gain and Specific Growth Rate (SGR)

Weight gain and specific growth rate (SGR) of shrimp varied significantly between treatments. The highest weight gain was observed in the shrimp fed the combination of Mannan-oligosaccharides (MOS), Fructo-oligosaccharides (FOS), Xylo-oligosaccharides (XOS), and Probiotic strains added including *Lactobacillus rhamnorus & Bacillus licheniformis* (T_{12}). These shrimp showed a substantial increase in body weight, with a final average weight of 16.38 g per shrimp at the end of the 60 day feeding trail experimental period, which was significantly higher than all other treatments. Specifically, the combination of MOS, FOS, and XOS with the probiotic demonstrated synergistic effects that led to superior growth performance compared to individual treatments. In terms of SGR, shrimp in T_{12} exhibited the highest rate (2.12%/day), significantly outperforming all other groups. This result was followed by the MOS + FOS + Probiotic (T_9) and MOS + XOS + Probiotic (T_{10}) and FOS + XOS + Probiotic (T_{11}) treatments, with SGR values of 2.04%/day. The control diet (T_1) and probiotic alone (T_2) showed the lowest SGR, at 1.75%/day and 1.84%/day, respectively. Our present study illustrated that growth performance (WG and SGR) and feed utilization (PER) were found to be significantly improved in shrimps fed with different experimental diets formulated with both probiotics and prebiotics. Although other studies have evaluated the use of symbiotics, this is the first to investigate symbiotics in the in the form of combinations of prebiotics including MOS, FOS, XOS with probiotics *L.rhamnorus* and *B.licheniformis*. Our results gains support from earlier reports that prebiotics shows significantly positive effect on growth performance in *L.vannamei* (13).

The results of this study clearly demonstrate the positive influence of dietary supplementation with probiotics and prebiotics—specifically mannan-oligosaccharides (MOS), fructo-oligosaccharides (FOS), xylo-oligosaccharides (XOS), and their combinations—on the growth performance of *Litopenaeus vannamei*. Among the various treatment groups, those receiving combined formulations of probiotics and prebiotics showed a significantly higher weight gain and specific growth rate (SGR) when compared to control and individual supplementation groups.

Weight Gain

The inclusion of prebiotics such as MOS, FOS, and XOS individually resulted in moderate improvements in weight gain relative to the control, which is consistent with previous studies indicating their role in enhancing gut health and nutrient absorption. MOS, derived from yeast cell walls, is known to bind pathogenic bacteria, thereby reducing gut inflammation and improving intestinal morphology. FOS and XOS, by promoting beneficial microbial populations such as *Lactobacillus* and *Bifidobacterium*, enhance fermentation and the production of short-chain fatty acids, leading to improved energy utilization.

However, the synergistic effects were most pronounced in the groups supplemented with a combination of probiotics and prebiotics. The enhanced weight gain in these groups suggests a prebiotic-probiotic interaction that optimizes gut microbiota composition and functionality. This synbiotic effect likely promotes better nutrient assimilation and immune modulation, which translate into enhanced somatic growth. The highest weight gain was observed in the group receiving a combined formulation of probiotics + XOS, FOS & MOS indicating that XOS, MOS & FOS may provide a particularly favourable substrate for the probiotic strains used.

Specific Growth Rate (SGR)

SGR, a reliable metric for evaluating overall growth efficiency, followed a trend similar to that of weight gain. The control group consistently exhibited the lowest SGR, highlighting the necessity of functional feed additives to optimize shrimp growth under intensive culture conditions. Among the experimental groups, the synbiotic treatments exhibited statistically significant ($p < 0.05$) increases in SGR values compared to both the control and monotypic treatments. The enhanced SGR in synbiotic treatments may be attributed to several mechanisms: improved feed digestibility due to enhanced enzymatic activity, reduced energy expenditure on immune defence due to stabilized gut microbiota, and improved intestinal morphology allowing for greater nutrient uptake. Notably, the combination of probiotics with XOS yielded the highest SGR, followed closely by combinations with FOS and MOS, suggesting that XOS may possess superior ferment ability and selectivity for probiotic organisms in shrimp intestines.

Feed Conversion Ratio (FCR)

The feed conversion ratio (FCR) was calculated to assess the efficiency of feed utilization by the shrimp. A lower FCR indicates more efficient use of the feed for growth. The shrimp fed the combination of MOS, FOS, XOS, and Probiotic added including *Lactobacillus rhamnorus & Bacillus licheniformis* (T_{12}) exhibited the most efficient feed conversion, with an FCR of 1.42. This was significantly lower than all other treatments, suggesting that the combination of probiotics and prebiotics enhanced the shrimp's ability to convert feed into body mass efficiently. In contrast, the control group (T_1) and probiotic alone group (T_2) showed higher FCR values (2.38 and 1.78, respectively), indicating less efficient feed conversion. The FCR values for treatments containing individual prebiotics (T_3, T_4, T_5) ranged from 1.77 to 1.79, reflecting moderate feed conversion efficiency.

Feed Conversion Ratio (FCR) is a critical parameter in aquaculture used to evaluate the efficiency with which feed is converted into biomass. In this study, the administration of probiotics and prebiotics, both individually and in combination, demonstrated significant effects on the FCR of *Litopenaeus vannamei*, indicating improved feed utilization and growth performance. Shrimp fed diets supplemented with individual prebiotics—Mannan-oligosaccharides (MOS), Fructo-oligosaccharides (FOS), and Xylo-oligosaccharides (XOS)—showed a moderate improvement in FCR compared to the control group. The improvement in FCR can be attributed to the enhancement of gut morphology, increased digestive enzyme activity, and modulation of the intestinal microbiota, which together optimize nutrient absorption. However, the most pronounced improvement in FCR was observed in groups receiving synbiotic formulations (probiotic + prebiotic combinations). Among these, the combination of probiotics with FOS exhibited the lowest FCR value (1.53), followed closely by MOS and XOS (1.54) combinations. The synergistic action is likely due to the selective stimulation of beneficial gut bacteria by the prebiotics, which, in the presence of exogenous probiotic strains, resulted in a more stable and functionally efficient gut microbiome. This symbiotic relationship may have reduced energy expenditure in immune responses and enhanced metabolic efficiency, allowing more energy to be allocated toward growth.

Statistical analysis confirmed that the reduction in FCR in the synbiotic groups was significant ($p < 0.05$) when compared with the control and individual treatment groups. These findings align with previous reports indicating that synbiotics not only improve growth performance but also enhance feed efficiency by promoting better gut health and nutrient assimilation (ref). Furthermore, lower FCRs suggest a sustainable advantage in commercial shrimp farming, reducing feed costs and minimizing waste output. This is particularly relevant in intensive culture systems where feed constitutes the major operational cost. In summary, the study highlights that synbiotic supplementation, particularly the combination of probiotics with FOS, yields the most efficient feed utilization in *L. vannamei*, supporting its application in commercial aquaculture for improved productivity and sustainability.

Survival Rate

Survival rate is an important parameter in aquaculture as it reflects the overall health and robustness of the cultured species. The highest survival rate was observed in the shrimp fed the MOS + FOS + XOS + Probiotic diet (T_{12}), with a survival rate of 94.6%. This group showed excellent resilience, with minimal mortality observed throughout the experimental period. Similarly, the groups supplemented with probiotics (T_2, T_6, T_7, T_8, T_9, T_{10}, T_{11}, T_{12}) exhibited significantly higher survival rates (ranging from 88.2% to 94.6%) compared to the control group (T_1), which had a survival rate of 83.5%. This suggests that the addition of probiotics, either alone or in combination with prebiotics, improves shrimp survival, likely by enhancing immunity and reducing susceptibility to stress and disease.

Statistical Analysis

The data were subjected to two-way ANOVA to determine the effects of probiotic and prebiotic supplementation on the growth performance of *L. vannamei*. The results indicated that both the probiotic added including *Lactobacillus rhamnorus & Bacillus licheniformis* and prebiotic supplementation had significant effects on all measured parameters ($p < 0.05$). The combination of probiotics and prebiotics (MOS, FOS, XOS) led to a significant improvement in shrimp growth, FCR, and survival rate compared to the control diet and individual prebiotic treatments. Post-hoc analysis using Tukey's HSD test revealed significant differences in growth parameters between treatments, with the T_{12} group (MOS + FOS + XOS + Probiotic) outperforming all other treatments, followed by T_9 (MOS + FOS + Probiotic), T10 (MOS + XOS + Probiotic), and T_6 (MOS + Probiotic). The control diet (T_1) had the lowest growth and survival parameters compared to other feeding treatments.

The results of this study provide compelling evidence that the combination of probiotics and prebiotics can significantly enhance the growth performance, feed efficiency, and survival rate of *Litopenaeus vannamei* when compared to control diets or single component supplementation. Our findings are consistent with previous studies that have highlighted the positive effects of probiotics and prebiotics on various aquaculture species, including shrimp, indicating that the integration of both functional feed additives can synergistically improve overall aquaculture productivity.

Synergistic Effects of Probiotics and Prebiotics

The primary aim of this study was to explore the synergistic effects of probiotics added including *Lactobacillus rhamnorus & Bacillus licheniformis* and three types of prebiotics (MOS, FOS, and XOS) on shrimp growth, feed utilization, and survival. The results demonstrate that the combination of all three prebiotics (MOS, FOS, XOS) with probiotics (T_{12}) resulted in the highest growth parameters, including weight gain (15.50 g), specific growth rate (2.12%/day), and the lowest feed conversion ratio (FCR) (1.42). This highlights the potential of combining multiple prebiotics with probiotics to optimize shrimp culture operations. Prebiotics, such as MOS, FOS, and XOS, serve as non-digestible ingredients that selectively promote the growth and activity of beneficial microorganisms in the gut. MOS is known for its ability to bind to pathogenic bacteria, reducing their adhesion to the gut lining and promoting a healthier microbiota. FOS and XOS, on the other hand, serve as substrates for beneficial microorganisms like bifidobacteria and lactobacilli, improving gut health and digestibility. The probiotics including *Lactobacillus rhamnorus & Bacillus licheniformis* added in this study likely contributed to maintaining a balanced gut microbiota, enhancing the shrimp's ability to digest feed efficiently, and bolstering their immune systems. This combination of prebiotics and probiotics in T_{12} could have led to a more favorable intestinal environment that maximized nutrient absorption, thus promoting faster growth and better feed conversion. The synergistic effects of prebiotics and probiotics have been demonstrated in other studies with various aquatic species. For example, in studies with fish [10, 15] and *Penaeus monodon* [6], L.vannamei [11, 12] and Cray fish [5] similar beneficial effects on growth and immune modulation were reported when these additives were combined. In our study, the significant improvement in growth parameters and FCR in the T_{12} group supports the hypothesis that prebiotics and probiotics, when used together, create a synergistic effect that enhances the digestive health and growth of shrimp.

Individual Effects of Probiotics and Prebiotics

While the combination of probiotics and prebiotics (T_{12}) performed best, the individual treatments also exhibited varying degrees of improvement compared to the control diet. For example, the group receiving only the probiotic added including *Lactobacillus rhamnorus & Bacillus licheniformis* (T_2) showed significant improvements in weight gain, specific growth rate, and FCR over the control group. This indicates that probiotics alone can positively influence shrimp growth by enhancing gut microbiota, digestibility, and possibly immunological defense mechanisms. Among the prebiotics, MOS supplementation (T_3) resulted in significant growth improvements over the control, with a weight gain of 10.29 g and an SGR of 1.84%/day. MOS has been previously recognized for its ability to enhance immune responses and reduce pathogen load in shrimp by promoting beneficial gut bacteria. This is consistent with findings from other aquaculture studies where MOS supplementation improved survival and growth performance, likely due to its prebiotic effects on beneficial gut microorganisms. Similarly, FOS (T_4) and XOS (T_5) also improved growth, though not to the same extent as the combined treatments, suggesting that individual prebiotics can have positive, but less pronounced, effects on shrimp culture. In particular, XOS supplementation (T_5) was found to enhance growth and feed conversion when compared to the control, which is in line with previous research on the role of xylo-oligosaccharides in stimulating beneficial gut microbiota in aquaculture species. XOS has been noted for its ability to promote the growth of Lactobacillus and Bifidobacterium, which are crucial for maintaining gut health and digestion efficiency [16].

Feed Conversion Efficiency and Immune System Modulation

One of the key findings of this study was the improvement in feed conversion efficiency (FCR) with probiotic and prebiotic supplementation. The T_{12} group demonstrated the best feed conversion, with an FCR of 1.42, which suggests that the combination of probiotics and prebiotics enhanced the shrimp's ability to convert feed into body mass effectively. This result is significant from an economic perspective, as improving FCR leads to reduced feed costs and increased production efficiency in aquaculture systems. The improvement in FCR is likely attributable to better nutrient absorption and utilization, enhanced by the beneficial gut microbiota promoted by the probiotics and prebiotics. Several studies have suggested that the probiotics used in aquaculture can enhance feed utilization by producing digestive enzymes, modulating intestinal motility, and reducing pathogenic bacteria. In this study, the probiotics including *Lactobacillus rhamnorus & Bacillus licheniformis* are likely contributed to these mechanisms, as it is known to produce lactic acid and antimicrobial peptides that inhibit harmful gut pathogens while promoting beneficial microbiota. The presence of prebiotics further enhances these effects by providing a selective substrate for the growth of beneficial microbes. Moreover, the improved survival rate observed in the probiotic-prebiotic treatments (T_6 to T_{12}) points to enhanced immunological responses. Probiotics are well known for their immune-stimulatory properties, which can strengthen the shrimp's innate immunity, making them more resilient to diseases and environmental stress. The synergistic action of prebiotics and probiotics may have further enhanced these effects, as prebiotics such as MOS have been shown to stimulate immune system components like hemocytes and enhance phagocytic activity in shrimp (Unpublished data). This immune boost likely contributed to the higher survival rates observed in the treatment groups, particularly in T_{12}, which had the highest survival rate (94.6%).

Comparison to Control and Probiotic Alone Diet

The control diet (T_1) and the probiotic alone diet (T_2) demonstrated relatively lower growth rates and higher FCR compared to the supplemented diets, particularly those that combined probiotics with prebiotics. This suggests that while probiotics alone offer some benefits to shrimp growth and feed conversion, their full potential is realized when combined with prebiotics. The combination of prebiotics and probiotics likely facilitates a more balanced and healthy gut microbiota, which is essential for optimal nutrient digestion, immune function, and overall shrimp health. The use of probiotics in aquaculture has grown in popularity due to their potential to improve feed utilization and reduce disease incidence. However, the addition of prebiotics to probiotic-supplemented diets has been shown to enhance the efficacy of probiotics by selectively promoting beneficial microbes in the gut. This combination of probiotic and prebiotic supplementation has proven to be more effective than either additive used alone in enhancing growth, FCR, and survival rate in shrimp culture. The results of this study have important implications for commercial shrimp farming. The combination of MOS, FOS, XOS, and probiotics added including *Lactobacillus rhamnorus & Bacillus licheniformis* can be utilized to enhance shrimp growth, feed efficiency, and survival, which are critical factors for the profitability and sustainability of shrimp farming operations. By improving these performance parameters, farmers can reduce feed costs, increase production efficiency, and enhance shrimp health, leading to more sustainable aquaculture practices. Moreover, the high survival rates observed in the probiotic-prebiotic supplemented groups suggest that these dietary strategies could also help mitigate the risks of disease outbreaks, a major challenge in shrimp farming. The use of probiotics and prebiotics could provide a natural, cost-effective alternative to antibiotics, which are increasingly being phased out due to concerns over antimicrobial resistance and environmental impact.

Given the growing demand for sustainable and environmentally friendly aquaculture practices, this research holds significant promise in advancing the development of nutritionally optimized shrimp diets that can improve farm productivity, reduce dependency on antibiotics, and promote sustainable aquaculture practices. Ultimately, the findings of this study may offer valuable insights into the potential applications of probiotics and prebiotics in shrimp farming, contributing to the long-term success and resilience of the aquaculture industry.

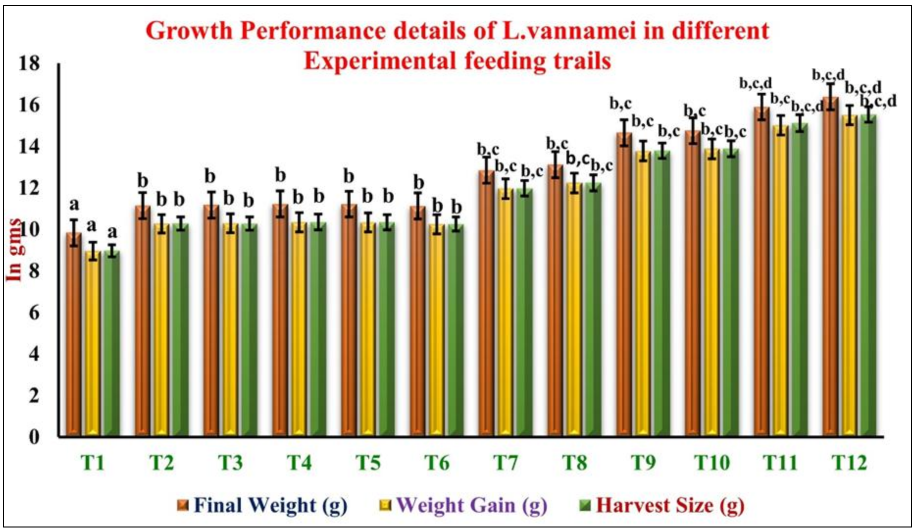
Table 1: Ingredient Composition of Control Experimental diet (Protein content 35%)

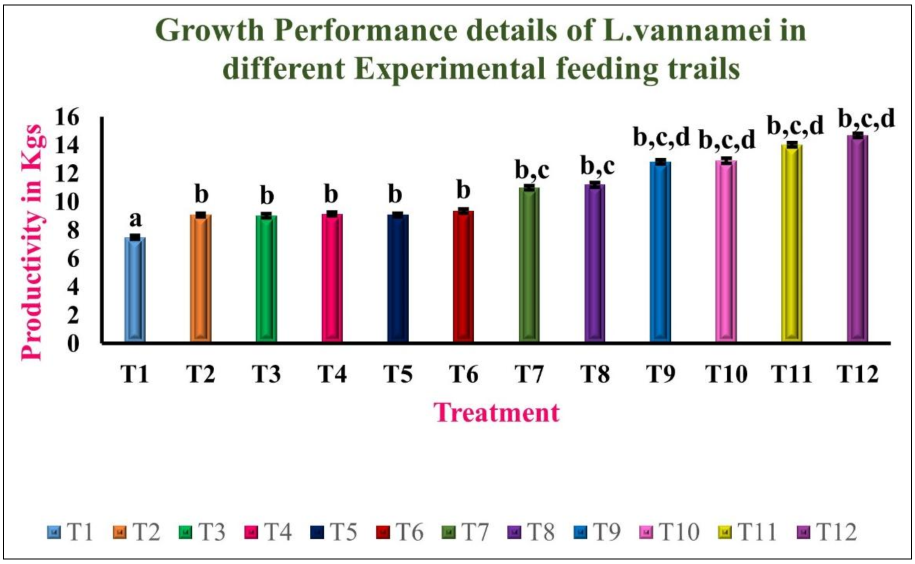
Feed Ingredient	(%)
Shrimp meal	15
Squilla meal	12
Soya bean meal	20
Wheat meal	20
Yeast meal	5
Groundnut oil cake	5
Cod liver oil	5
Vegetable oil	4
Ascorbic acid	2
Choline chloride	1
Vitamin mixture	1
Mineral mixture	1
Chromic oxide	1
Agar Agar	3
Gelatin	5
Total	100

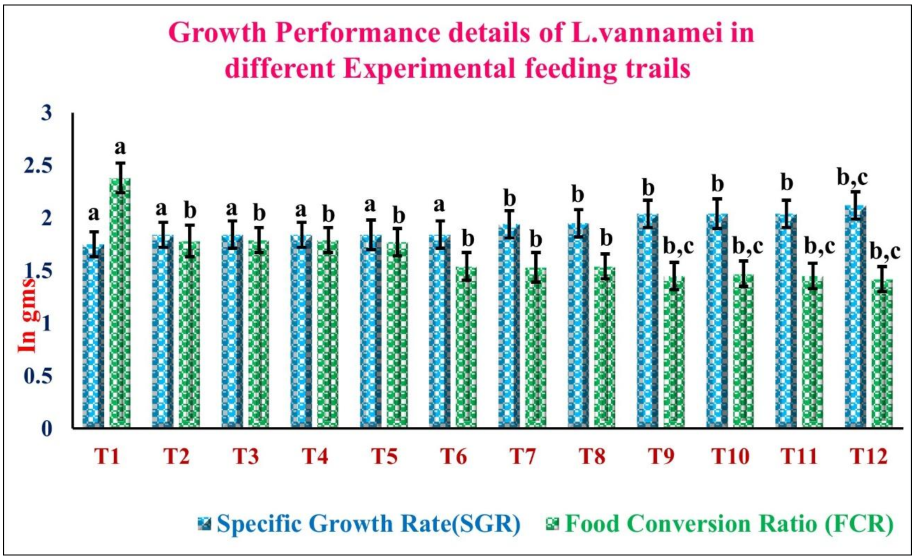
Table 2: Growth Performance details of *L.vannamei* in different Experimental feeding trails

		T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
Shrimp Stocked(No's)		1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Experimental Period (Days)		60	60	60	60	60	60	60	60	60	60	60	60
Per cent Survival (%)		83.5^{a}	88.2^{a}	87.7^{a}	88.2^{a}	87.7^{a}	91.2^{b}	91.7^{b}	91.4^{b}	93.1^{b}	92.9^{b}	92.7^{b}	94.6^{b}
		± 1.2	± 1.1	± 1.2	± 1.3	± 1.2	± 1.2	± 1.2	± 1.3	± 1.2	± 1.2	± 1.2	± 1.3
Final Weight (g)		9.83^{a}	11.14^{b}	11.17^{b}	11.22^{b}	11.21^{b}	11.12^{b}	12.84^{b,c}	13.11^{b,c}	14.65^{b,c}	14.75^{b,c}	15.89^{b,c,d}	16.38^{b,c,d}
		±0.52	±0.61	±0.56	±0.57	±0.61	±0.55	±0.57	±0.57	±0.59	±0.64	±0.65	±0.72
		PDC	+13.33	(+13.63)	(+14.14)	(+14.04)	(+13.12)	(+30.62)	(+33.37)	(+49.04)	(+50.01)	(+61.65)	(+66.63)
Weight Gain (g)		8.95^{a}	10.26^{b}	10.29^{b}	10.34^{b}	10.33^{b}	10.24^{b}	11.96^{b,c}	12.23^{b,c}	13.77^{b,c}	13.87^{b,c}	15.01^{b,c}	15.5^{b,c,d}
		±0.43	±0.44	±0.45	±0.46	±0.45	±0.46	±0.47	±0.47	±0.48	±0.48	±0.48	±0.47
		PDC	(+14.64)	(+14.97)	(+15.53)	(+15.42)	(+14.41)	(+33.63)	(+36.65)	(+53.85)	(+54.97)	(+67.71)	(+73.18)
Daily Growth Rate (g)		0.149^{a}	0.171^{b}	0.172^{b}	0.173^{b}	0.172^{b}	0.171^{b}	0.199^{b,c}	0.204^{b,c}	0.230^{b,c,d}	0.231^{b,c,d}	0.251^{b,c,d}	0.258^{b,c,d}
		±0.015	±0.017	±0.017	±0.016	±0.017	±0.016	±0.017	±0.018	±0.018	±0.018	±0.019	±0.019
		PDC	(+14.76)	(+15.44)	(+16.11)	(+15.44)	(+14.76)	(+33.56)	(+36.91)	(+54.36)	(+55.04)	(+68.46)	(+73.15)
Daily Growth Rate (%)		24.84^{a}	28.51^{b}	28.67^{b}	28.84^{b}	28.67^{b}	28.51^{b}	33.17^{b,c}	34.01^{b,c}	38.34^{b,c}	38.51^{b,c}	41.84^{b,c}	43.01^{b,c,d}
		±1.23	±1.28	±1.29	±1.28	±1.27	±1.25	±1.29	±1.32	±1.33	±1.34	±1.35	±1.35
		PDC	(+14.77)	(+15.42)	(+16.10)	(+15.42)	(+14.75)	(+33.53)	(+36.91)	(+54.34)	(+55.03)	(+64.44)	(+73.15)
Daily Growth Index (DGI)		4.98^{a}	5.71^{b}	5.72^{b}	5.75^{b}	5.74^{b}	5.69^{b}	6.65^{b,c}	6.8^{b,c}	7.66^{b,c}	7.71^{b,c}	8.34^{b,c,d}	8.62^{b,c,d}
		±0.12	±0.14	±0.13	±0.14	±0.13	±0.14	±0.14	±0.14	±0.15	±0.15	±0.16	±0.15
		PDC	(+14.66)	(+14.86)	(+15.46)	(+15.26)	(+14.26)	(+33.53)	(+36.55)	(+53.82)	(+54.82)	(+67.47)	(+73.09)
Specific Growth Rate(SGR)		1.75^{a}	1.84^{a}	1.84^{a}	1.84^{a}	1.84^{a}	1.84^{a}	1.94^{b}	1.95^{b}	2.04^{b}	2.04^{b}	2.04^{b}	2.12^{b,c}
		±0.12	±0.12	±0.13	±0.12	±0.14	±0.13	±0.13	±0.13	±0.13	±0.14	±0.13	±0.13
		PDC	(+5.14)	(+5.14)	(+5.14)	(+5.14)	(+5.14)	(+10.86)	(+11.43)	(+16.57)	(+16.57)	(+16.57)	(+21.14)
Food Conversion Ratio (FCR)		2.38^{a}	1.78^{b}	1.79^{b}	1.79^{b}	1.77^{b}	1.54^{b}	1.53^{b}	1.54^{b}	1.45^{b,c}	1.47^{b,c}	1.45^{b,c}	1.42^{b,c}
		±0.14	±0.15	±0.12	±0.12	±0.13	±0.13	±0.14	±0.12	±0.13	±0.12	±0.12	±0.12
		PDC	(-25.21)	(-24.79)	(-26.05)	(-25.63)	(-35.29)	(-35.71)	(-35.29)	(-39.08)	(-38.24)	(-39.08)	(-40.34)
Feed Efficiency Ratio (FER)		0.42^{a}	0.56^{b}	0.56^{b}	0.57^{b}	0.57^{b}	0.65^{b,c}	0.65^{b,c}	0.65^{b,c}	0.69^{b,c}	0.68^{b,c}	0.69^{b,c}	0.70^{b,c}
		±0.05	±0.05	±0.06	±0.05	±0.07	±0.06	±0.06	±0.06	±0.06	±0.06	±0.06	±0.06
		PDC	(+33.33)	(+33.33)	(+35.71)	(+35.71)	(+54.76)	(+54.76)	(+54.76)	(+64.29)	(+61.90)	(+64.29)	(+66.67)
Feed Conversion Efficiency (%)		42^{a}	56^{b}	56^{b}	57^{b}	57^{b}	65^{b,c}	65^{b,c}	65^{b,c}	69^{b,c}	68^{b,c}	69^{b,c}	70^{b,c}
		±2	±3	±2	±3	±3	±3	±3	±3	±3	±3	±3	±3
		PDC	(+33.33)	(+33.33)	(+35.71)	(+35.71)	(+54.76)	(+54.76)	(+54.76)	(+64.29)	(+61.90)	(+64.29)	(+66.67)
Protein Efficiency Ratio (PER)		5.48^{a}	6.78^{b}	7.32^{b}	7.49^{b}	7.62^{b}	7.63^{b}	7.74^{b}	8.13^{b}	8.24^{b,c}	8.39^{b,c}	8.52^{b,c}	8.54^{b,c}
		±0.22	±0.26	±0.27	±0.27	±0.28	±0.29	±0.28	±0.29	±0.28	±0.29	±0.29	±0.31
		PDC	(+23.72)	(+33.58)	(+36.68)	(+39.05)	(+39.23)	(+41.24)	(+48.36)	(+50.36)	(+50.10)	(+55.47)	(+55.84)
Harvest Size (g)		8.97^{a}	10.28^{b}	10.27^{b}	10.35^{b}	10.34^{b}	10.25^{b}	11.97^{b,c}	12.24^{b,c}	13.78^{b,c}	13.88^{b,c}	15.12^{b,c,d}	15.53^{b,c,d}
		±0.29	±0.32	±0.32	±0.37	±0.36	±0.34	±0.38	±0.39	±0.37	±0.39	±0.41	±0.38
		PDC	(+14.60)	(+14.49)	(+15.38)	(+15.27)	(+14.27)	(+33.44)	(+36.45)	(+53.62)	(+54.74)	(+68.56)	(+73.13)
Shrimp Count (No's/kg)		111^{a}	97^{a}	97^{a}	96^{a}	96^{a}	97^{a}	83^{b}	81^{b}	72^{b}	72^{b}	66^{b}	63^{b,c}
		±3	±2	±2	±2	±3	±2	±3	±2	±2	±2	±2	±2
		PDC	(-12.61)	(-12.61)	(-13.51)	(-13.51)	(-12.61)	(-25.23)	(-27.03)	(-35.13)	(-35.13)	(-40.54)	(-43.24)
Productivity (kgs)		7.49^{a}	9.07^{b}	9.01^{b}	9.13^{b}	9.07^{b}	9.35^{b}	10.98^{b,c}	11.19^{b,c}	12.83^{b,c,d}	12.89^{b,c,d}	14.02^{b,c,d}	14.69^{b,c,d}
		±0.12	±0.14	±0.14	±0.12	±0.12	±0.14	±0.15	±0.16	±0.15	±0.17	±0.15	±0.14
		PDC	(+21.09)	(+20.29)	(+21.90)	(+21.09)	(+24.83)	(+46.60)	(+49.40)	(+71.30)	(+72.10)	(+87.19)	(+96.13)

Initial Weight 0.88±0.06 g
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 @ $p < 0.05$







Conclusion on Synergistic Effects of Probiotics and Prebiotics: The results from the present study underscore the significant synergistic effects of combining prebiotics (MOS, FOS, XOS) with probiotics added including *Lactobacillus rhamnorus & Bacillus licheniformis* in the aquaculture of *Litopenaeus vannamei*. Each treatment involving probiotics and prebiotics led to notable improvements in shrimp growth, feed conversion efficiency, and overall health, which were more pronounced than those seen in the control group or the individual probiotic or prebiotic treatments. This demonstrates that the combined use of probiotics and prebiotics can work synergistically to enhance shrimp culture operations, offering a holistic approach to improving shrimp performance in aquaculture systems.

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