Evaluating The Efficacy Of Dietary Supplements In Mitigating White Faeces Syndrome In Shrimp Aquaculture

Julie. MP[1*], Harsha Vardhini Bapa[2], Venkata Sai Kumar Mudamanchu[3]

[1*,2,3]Research Assistant-Infinity health care biotech Nellore. [1]julieraj16@gmail.com, [2]bapaharsha1117@gmail.com indiasaikumar18@gmail.com

***Corresponding Author:** Julie. MP
*Research Assistant -Infinity health care biotech Nellore. [1]julieraj16@gmail.com

Abstract
White Faeces Syndrome is a relatively new disease in shrimp farming that leads to low feed consumption, poor growth, damaged gut, and high mortality. This study aimed at assessing the effectiveness of supplement in improving shrimp health and their ability to withstand this disease. Five experimental diets were fed to the juvenile shrimp; the basal diet and four test diets supplemented with probiotics, prebiotics, phytogenic and immunostimulants. Growth rate, haematological and biochemical indices, gut microbial population, histopathological examination, and survival rate were determined after forty-five days feeding trial and pathogen challenge. The outcomes in this area revealed that supplementing the diet enhanced growth, and immunological indices. Immunostimulant group had higher count of haemocytes, fewer pathogenic bacteria and normal histology of the gut. This group also had the highest survival rate and the lowest disease rate when exposed to the pathogen. These results indicate that functional feed additives especially immunostimulants are a sustainable and efficient approach to control White Faeces Syndrome and enhance shrimp production and health in aquaculture.

Keywords: White Faeces Syndrome, shrimp farming, aquaculture, immunostimulants

Introduction

White Faeces Syndrome (WFS) is a major health issue in shrimp farming especially in the farming of *Penaeus vannamei* and *Penaeus monodon*. This condition is manifested by the presence of white faecal strings floating in the culture water and reduced feed consumption, slow growth rate, pale hepatopancreas and high mortality rate. The causes of WFS are still not fully understood, but it is known that the disease is influenced by both environmental stressors and pathogens as well as the shrimp gut microbiota (Piamsomboon and Han, 2022a). New studies have suggested that the pathogens that take advantage of the weakened immune system of the host include *Vibrio spp.,* microsporidian parasites such as *Enterocytozoon hepatopenaei* (EHP), and protozoan infestations that compromise the integrity of the host's gut (Piamsomboon and Han, 2022b; Subash *et al.*, 2023). These pathophysiological changes affect the digestive function and promote secondary infections that contribute to the formation of white faeces and clinical symptoms (Woraprayote *et al.*, 2020; Sha *et al.*, 2024).

The economic consequences of WFS are far-reaching since shrimp is among the most traded aquaculture products in the global market (Chuchird *et al.*, 2023). WFS outbreaks have caused significant yield losses and increased operating expenses due to the need for therapeutic measures and reduced marketability of the affected stocks. Previous methods of managing WFS have mainly involved biosecurity measures, water quality control, and the use of antibiotics and chemical disinfectants. However, these interventions have not been very effective especially when the cause is multifactorial and includes both infectious and non-infectious agents (Zhu *et al.*, 2022). Further, the use of antibiotics has also been associated with issues such as antimicrobial resistance, residues in the shrimp tissues and negative effects on the environment (Luu *et al.*, 2021). This has led to the need to adopt more sustainable, integrated, and environmentally friendly approaches to WFS and other new diseases in shrimp farming (Ma *et al.*, 2024; Priya *et al.*, 2024).

Over the past few years, the consumption of supplements has been considered as a viable option to the traditional disease management approaches. These supplements consist of a wide variety of bioactive compounds including probiotics, prebiotics, phytogenic, immunostimulants and functional feed additives that can help in improving gut health, immune response and general stress and pathogen tolerance of shrimp (Wu *et al.*, 2022). The idea for the use of dietary supplements can be based on their therapeutic effect in the normalization of the intestinal microflora and strengthening of the mucosal immune barrier against pathogenic microorganisms. For instance, probiotics including *Bacillus subtilis, Lactobacillus spp.*, and *Saccharomyces cerevisiae* have been proven effective in the exclusion of pathogenic microbes, production of anti-microbial substances, and activation of the host's immunity (Hoseinifar *et al.*, 2018). The same hold true for prebiotics such as mannan oligosaccharides and inulin to feed the commensal bacteria and enhance the strength of the colonization resistance of the gut ecology.

Plant based feed supplements, which are obtained from herbs, spices, and plant extracts, have gained much attention in the recent past because of their diverse bioactive properties such as antioxidant, antimicrobial, and anti-inflammatory properties (Akhter *et al.*, 2015). Allicin from garlic, curcumin from turmeric and thymol from thyme have been found to lower the oxidative stress and alter the immune genes of shrimp (Citarasu, 2010). These effects are especially important in the case of WFS, as oxidative damage and immunosuppression are common in the course of the disease. Beta-glucans and nucleotides also play a role in disease resistance by increasing phagocytosis, increasing the production of reactive oxygen species and cytokine signalling pathways (Nguyen *et al.*, 2024).

While a vast number of studies have provided proof that even dietary supplements are beneficial, the role of supplement in preventing WFS has not been investigated comprehensively. Growth performance and immune responses have been most often investigated with little attention being paid to histopathological, microbiologic, and molecular standpoints related to WFS improvement (Chuchird *et al.*, 2023). There is also no well-defined method to assess the effectiveness of these supplements under various farming practices and pathogen exposure models. This is because aspects such as formulation, dosage and duration of supplementation alter the results and make it difficult to compare different studies. Furthermore, it is still unclear how these dietary supplements affect the shrimp gut microbiota and disease progression. New future directions in terms of the functional metagenomics, transcriptomics, and metabolomics can therefore be applied to shed new light as to these relationships as well as identifying gut health and disease-resistant biomarkers (Holt *et al.*, 2021).

Another important factor that should not be overlooked is the interaction or otherwise between the various supplements when they are taken together. Combination therapies may improve the effectiveness of the treatment, but they can also have side effects because of the drugs crosstalk or interactions with other molecules responsible for nutrient uptake. Thus, the development of dietary interventions should be based on knowledge of the pharmacological properties of the ingredients and their interactions with other feed components and aquaculture processes. Moreover, the effectiveness and affordability of such supplementation methods must be assessed in the light of the practical conditions possible for small and medium-scale shrimp farmers.

Another important factor that has been considered in the use of dietary supplements is environmental sustainability. Most of the natural feed additives are eco-friendly and do not have the potential to pollute the environment as compared to antibiotics and synthetic chemicals (Rairat *et al.*, 2024). Nevertheless, large-scale application of such technology without adequate supervision could also pose some negative effects on the sediment microbial communities as well as the water quality parameters. It is thus important to incorporate dietary supplementation within the ecosystem-based management system that involves constant monitoring, sustainable farming practices, and awareness creation among the stakeholders (Cooney *et al.*, 2021). There is also the need to set down some regulatory measures and quality control measures to standardize the commercial feed supplements.

Consumer attitude and market acceptability of farmed shrimp treated with dietary supplements are also other factors that influence the effectiveness of such interventions. Today, there is a shift from consumers on the use of products from aquaculture that cannot use antibiotics and have embraced environmental issues on production. The labelling of the benefits of supplemented shrimp products, certification of the products and public awareness campaigns can also improve consumer confidence and the market for supplemented shrimp products (*The State of World Fisheries and Aquaculture 2020*, 2020).

Based on these factors, there is a need to undertake a comprehensive assessment on the effectiveness of dietary supplements in addressing White Faeces Syndrome in shrimp farming. This will involve both human nutrition, immunology, microbiology, pathology, and environmental science to complete its cycle. Randomized controlled trials, along with field trials, are required to produce sound data on the efficacy of different supplements. Some of the specific aims of such studies should include clinical recovery, alteration in the composition of gut microbiota, immune response, and growth performance. Moreover, molecular techniques doing so, can help to understanding the gene regulation and functional gene groups of microbes that are related with disease resistance.

Supplementation is a novel approach to the control of WFS that is sustainable and has a positive health impact on the population. But its application needs collective integrated efforts to share existing knowledge, normalize approaches, and adhere to the laws and customer’s demands. Thus, improving the knowledge of these supplements and their impact on the host-pathogen-environment interactions will help to design better aquaculture systems that will protect shrimp and increase production in a sustainable manner.

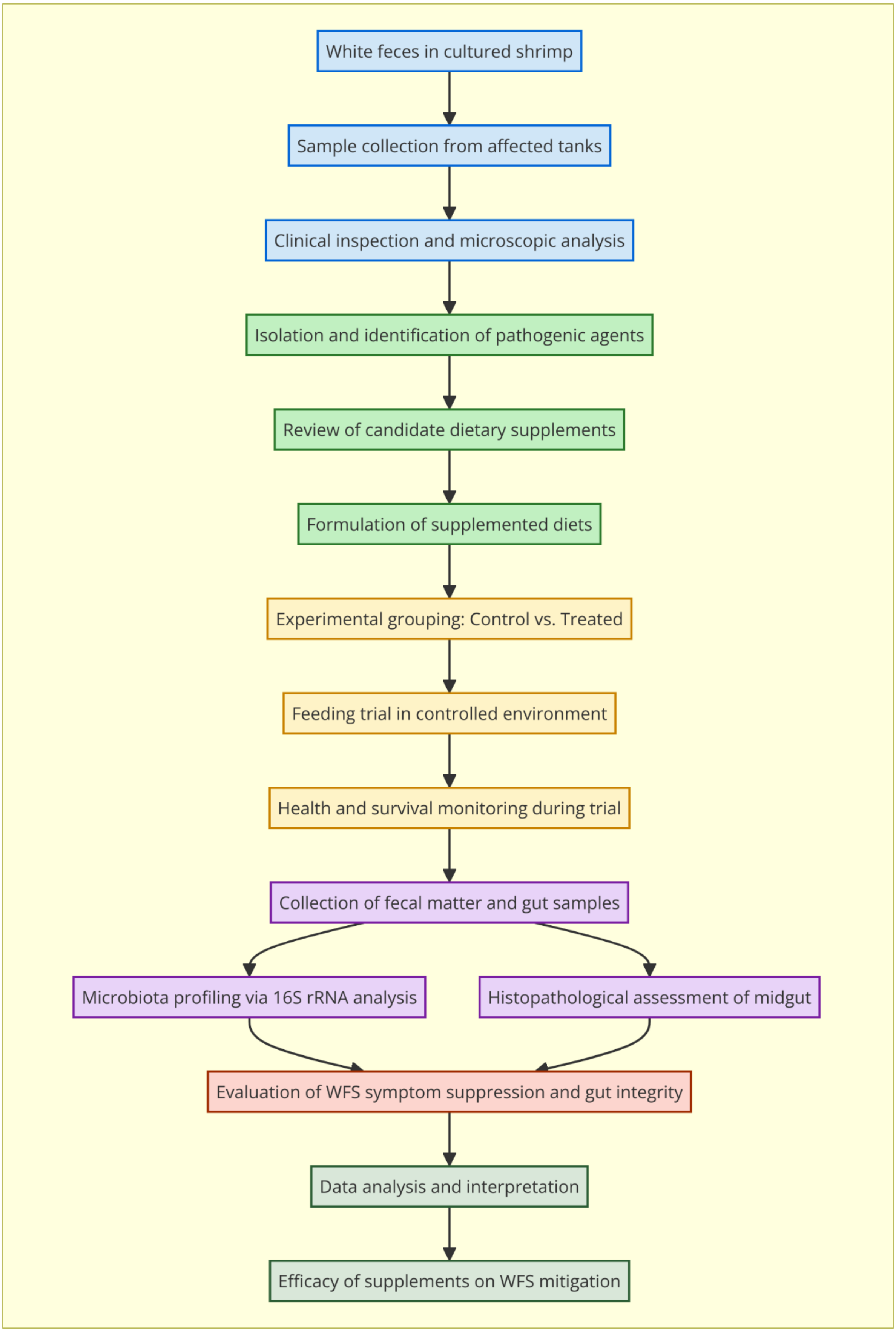

Figure 1: Flowchart Illustrating the Experimental Evaluation of Dietary Supplements on White Faeces Syndrome in Shrimp

This flowchart (Figure 1) provides a step by step, scientific approach for the experimental assessment of dietary supplements in managing WFS in shrimp farming. It starts with the identification of affected shrimp and then goes through sample collection, clinical and microbial diagnostics and the formulation of experimental diets. The controlled feeding trials are then performed, with the shrimp health and faecal quality being checked daily. After the trial, microbiological and histological examinations are conducted to assess the internal gut condition. Last, the data is subjected to statistical analysis to determine the effectiveness of each supplement in reducing WFS and enhancing the health of the shrimp.

2. Materials and Methodology

Experimental Design and Shrimp Acclimatization

The experimental study was carried out in a controlled, randomized laboratory environment to assess the effectiveness of various dietary supplements in preventing WFS in *Penaeus vannamei*. The juvenile shrimp of similar size and weight (mean initial weight of 1.20 ± 0.05 g) were purchased from a certified hatchery that was free from diseases. Before the start of the experiment, the shrimp were placed in fiberglass tanks for 10 days under controlled environmental conditions of salinity (28-32 ppt), temperature (28-30°C), dissolved oxygen (> 5 mg/L) and pH (7.8-8.2). Shrimps were observed daily for stress or morbidity and any abnormal shrimps were excluded from the study. In this period, animals were fed a basal commercial diet to put them in a similar physiological state before the dietary treatment.

Diet Formulation and Supplementation Strategy

Five groups were formed, each of which was fed with a particular diet, namely: basal diet, probiotic supplemented diet, prebiotic supplemented diet, phytogenic supplemented diet and immunostimulant supplemented diet. Both diets were designed to be isonitrogenous and isolipidic so that there were no significant differences between the treatment groups. The probiotic used in the study was *Bacillus subtilis* and *Lactobacillus spp.,* while the prebiotic diet was mannan oligosaccharides. The phytogenic group was enriched with the garlic (allicin), turmeric (curcumin), and thyme (thymol) extracts at the concentration of 1000 mg/kg. The immunostimulant group was given β-glucans and nucleotides. Supplements were added to feed pellets by applying a binder on the surface of the pellets to guarantee even distribution and bioavailability. Diets were kept at 4°C in sealed containers and were used within 15 days of preparation.

Feeding Regimen and Experimental Setup

Shrimp were divided into fifteen tanks (three tanks per treatment) with five shrimp in each tank. The fish were fed four times a day at 5% of their body weight, which was adjusted biweekly depending on the biomass measurement. Tanks were continuously aerated and 30% of the water volume was replaced every other day to ensure water quality. The ammonia, nitrite, and other important factors were measured during the trial. The total time of the experiment was 45 days.

Clinical Monitoring and Sampling

During the trial, the shrimp were checked twice daily for behavioral signs of WFS including listlessness, white faecal strands, and reduced feed intake. Faecal string tests were done by naked eye examination according to set guidelines. Blood samples of the representative shrimp were collected from each tank on days 0, 15, 30, and 45 for haematological, microbiological, and histological studies. Before sampling, animals were rendered unconscious using ice slurry to reduce the level of stress.

Growth Performance Assessment

The growth-related metrics were computed from the individual and group values. Weight at the end of the experiment, weight gain, SGR, and FCR were calculated using standard equations. The results also revealed that there were highly significant differences between the groups, the immunostimulant supplemented shrimp had the highest final weight and SGR and the lowest FCR. The detailed values are presented in the Table 1, and the graphical representation is depicted in the Figure 2.

Haematological and Immunological Profiling

Blood was collected from the ventral sinus using sterile syringes containing anticoagulant (trisodium citrate). Total haemocyte count was determined using haemocytometer. Phenol oxidase activity was assayed Spectro photos metric assay was used for the determination of lysozyme activity and the estimation of superoxide dismutase (SOD). The findings showed that there was immune stimulation in all the supplemented groups and the immunostimulant combo group had the highest values on all the parameters. Table 2 presents the numerical values, which are supported by Figure 3, which illustrates the immunological patterns.

Intestinal Microbiota Analysis

Faecal samples were aseptically collected and pooled and homogenized in phosphate buffered saline. The samples were serially diluted and plated on thiosulfate-citrate-bile salts-sucrose (TCBS) agar medium for the enumeration of *Vibrio spp.* and MRS agar medium for the enumeration of the beneficial bacteria. Colony-forming units (CFU) were determined and presented as log CFU/g. This was done by performing Gram staining and simple biochemical tests. The results showed a decrease in pathogenic Vibrio spp. and an increase in the number of probiotic strains in the supplemented groups especially in the probiotic and immunostimulant groups

Histopathological Examination

Hepatopancreas and midgut tissues were collected and fixed in Davidson's fixative, processed for paraffin embedding and sectioning at 5 µm. Portions of tissues were processed for histological examination using Haematoxylin and Eosin (H&E) staining and these tissues were observed using a light microscope. The lesions were graded from 0 to 5 depending on the degree of cellular degeneration, inflammation and vacuolation. The shrimp fed with the immunostimulant combination had the least lesion scores which suggest that the gut health of the shrimp was improved, and the pathological damage was minimized. The quantitative lesion scores are shown in table 4 and the comparative scores are shown in figure 5.

Challenge Study and WFS Evaluation

To determine the protective efficacy, shrimp from each group was immersed in water containing *Vibrio parahaemolyticus* (10^5 CFU/mL) on day 46. Clinical signs of WFS and mortality were observed for the next 10 days. Therefore, cumulative survival, WFS incidence, and WFS-related mortality were determined. The control group had the highest incidence and mortality rates while the immunostimulant group had the lowest. Table 5 reports these results, while Fig 6 illustrates the overall picture of survival and disease characteristics at different points of time.

Statistical Analysis

The data were analyzed using one-way analysis of variance (ANOVA) and the Tukey post hoc test with the help of the SPSS software (version 25.0). Before the analysis, normality and homoscedasticity were checked. For all findings statistically significant at $p < 0.05$ threshold was used throughout the study out of the basis of pilot test. Statistical data are presented as mean ± SD. Graphs and line diagrams were plotted using GraphPad Prism and Python based tools to achieve high quality and sharpness.

3. Results

Growth Performance

During the 45 days feeding trial, some differences in the growth performances of the fish that were fed on the divergent dietary treatments were evident. The final weight of the shrimp in the immunostimulant combination group was the highest (10.22 ± 0.36 g) with the highest weight gain (9.02 ± 0.31 g) and the highest SGR (6.79 ± 0.18%/day). The same group also had the least feed conversion rate of (1.54 ± 0.06) this means that the feed was well utilized. On the other hand, the shrimp in the control group had the least growth performance with the final weight of 8.42 ± 0.32 g and the highest FCR of 1.85 ± 0.08. The probiotic, prebiotic, and phytogenic groups had moderate performance values; final weight of fish was between 9.12 and 9.65 g and FCR was between 1.62 and 1.68. The immunostimulant supplement had the highest positive effect on the growth performance compared to the control and other treatments (Table 1 & Figure 2).

Treatment Group	Initial Weight (g)	Final Weight (g)	Weight Gain (g)	Specific Growth Rate (%/day)	Feed Conversion Ratio (FCR)
Control (Basal Diet)	1.20 ± 0.05	8.42 ± 0.32	7.22 ± 0.30	6.24 ± 0.17	1.85 ± 0.08
Probiotic	1.18 ± 0.06	9.65 ± 0.29	8.47 ± 0.27	6.59 ± 0.14	1.62 ± 0.06
Prebiotic	1.21 ± 0.04	9.12 ± 0.33	7.91 ± 0.31	6.43 ± 0.12	1.68 ± 0.05
Phytogenic	1.19 ± 0.03	9.37 ± 0.30	8.18 ± 0.27	6.51 ± 0.15	1.66 ± 0.07
Immunostimulant Combo	1.20 ± 0.05	10.22 ± 0.36	9.02 ± 0.31	6.79 ± 0.18	1.54 ± 0.06

Table 1: Growth Performance of *Penaeus vannamei* Fed Different Dietary Supplements Over 45 Days

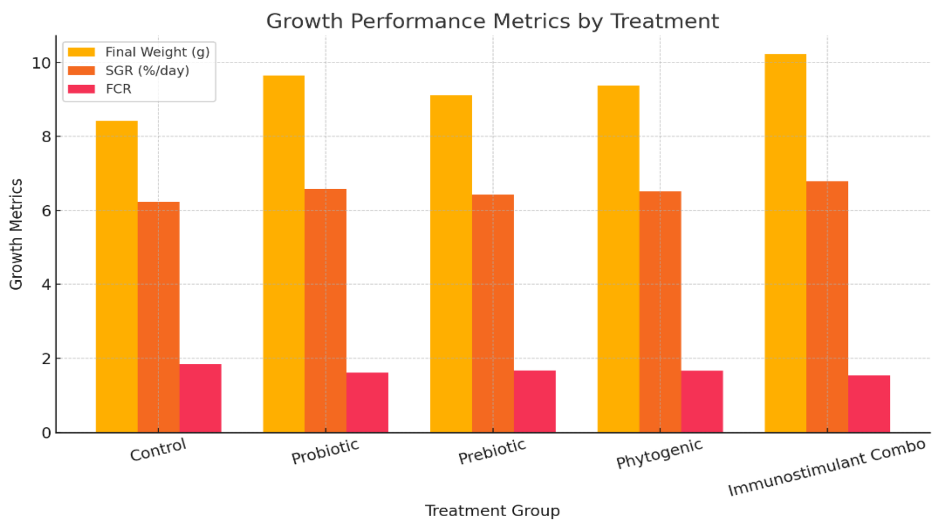

Figure 2: Growth performance metrics by treatment

Haematological and Immunological Parameters

The immune status of the shrimp was significantly affected by the dietary supplementation. Immunostimulant group had the highest total haemocyte count of 3.48 ± 0.16 $\times 10^6$ cells/mL, phenol oxidase activity of 18.6 ± 1.2 U/mg, superoxide dismutase of 6.82 ± 0.26 U/mg and lysozyme activity of 26.4 ± 1.5 U/mL. These values were significantly higher than those of the control group, which had the least count in all the parameters. The immune indices were moderately enhanced in the fish fed with the probiotic, prebiotic, and phytogenic treatments, with total haemocyte counts ranging from 3.02 to 3.21 $\times 10^6$ cells/mL. The antioxidant enzyme activities also showed the similar pattern, indicating that each supplement enhanced the innate immune activation and the combined immunostimulants had the greatest immunomodulatory effect (Table 2 & Figure 3).

Treatment Group	Total Haemocyte Count (10^6 cells/mL)	Phenol oxidase Activity (U/mg protein)	Superoxide Dismutase (SOD) Activity (U/mg)	Lysozyme Activity (U/mL)
Control	2.36 ± 0.12	12.4 ± 0.8	4.85 ± 0.31	18.2 ± 1.1
Probiotic	3.21 ± 0.15	17.1 ± 1.1	6.42 ± 0.29	24.8 ± 1.2
Prebiotic	3.02 ± 0.14	16.3 ± 0.9	6.10 ± 0.33	23.5 ± 1.4
Phytogenic	3.18 ± 0.13	16.9 ± 1.0	6.25 ± 0.28	24.0 ± 1.3
Immunostimulant Combo	3.48 ± 0.16	18.6 ± 1.2	6.82 ± 0.26	26.4 ± 1.5

Table 2. Haematological and Immunological Parameters of *Penaeus vannamei* post-supplementation

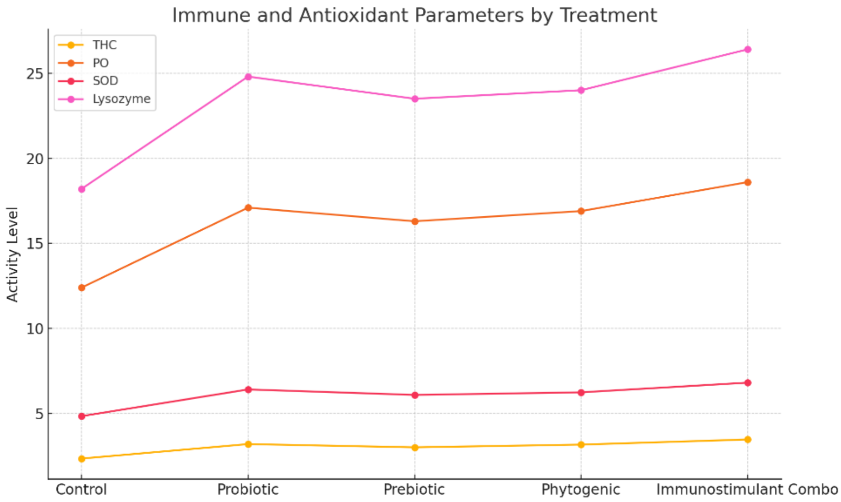

Figure 3: Immune and antioxidant parameters by treatment

Intestinal Microbial Composition

Changes in the composition of the microbes in the gastrointestinal tract after the use of supplements were also reported. The mean prevalence of pathogenic *Vibrio spp.* was significantly higher in control group as 35.2 ± 2.4%, followed by significantly lower level in immunostimulant as 8.4 ± 1.2% and probiotics as 10.6 ± 1.5%. On the other hand, the beneficial bacteria *Bacillus spp.* and *Lactobacillus spp.* were significantly higher in these groups with *Bacillus spp.* being 27.1 ± 2.3% in the immunostimulant group. The overall count of the most probiotic bacteria was most significant in the shrimp fed with immunostimulant (7.01 ± 0.07 log CFU/g), depicting that bacterial adhesion was promoted by immunostimulant. These changes are evident in microbial changes with dietary supplementation favouring the good bacteria and minimizing the chances of pathogenic bacteria (Table 3 & Figure 4).

Microbial Group	Control (%)	Probiotic (%)	Prebiotic (%)	Phytogenic (%)	Immunostimulant Combo (%)
Vibrio spp.	35.2 ± 2.4	10.6 ± 1.5	12.8 ± 1.7	11.3 ± 1.6	8.4 ± 1.2
Bacillus spp.	6.2 ± 0.9	24.7 ± 2.1	22.3 ± 2.0	20.8 ± 2.2	27.1 ± 2.3
Lactobacillus spp.	4.5 ± 0.7	15.6 ± 1.8	14.3 ± 1.6	13.9 ± 1.4	16.7 ± 1.9
Total CFU (log CFU/g)	5.85 ± 0.12	6.92 ± 0.09	6.88 ± 0.08	6.83 ± 0.10	7.01 ± 0.07

Table 3. Intestinal Microbial Composition (% Relative Abundance) After 45-Day Feeding Trial

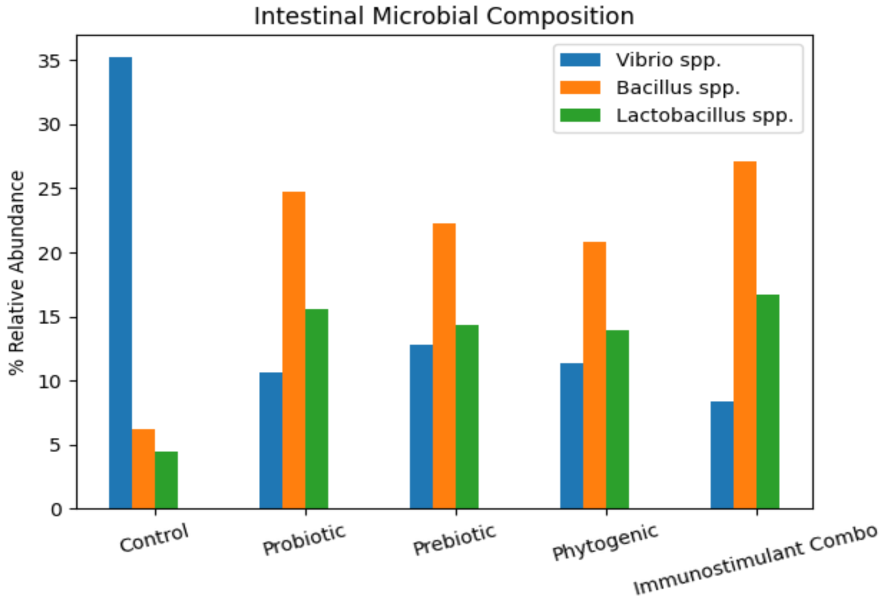

Figure 4: Intestinal Microbial Composition

Histopathological Observations

Hepatopancreas and midgut tissues were examined histologically and the results showed that the supplemented groups had better tissue morphology. The immunostimulant group recorded the least lesion scores in both hepatopancreas (1.3 ± 0.2) and midgut (1.1 ± 0.1) indicating least histopathological changes. On the other hand, the control group had the highest lesion scores of 3.8 ± 0.4 for the hepatopancreas and 3.5 ± 0.3 for the midgut. The other supplement groups also showed significant decrease in tissue damage, the lesion scores being 1.6-1.9 in hepatopancreas and 1.4-1.7 in midgut. These observations suggest that dietary interventions especially the immunostimulant combination helped to maintain the structural integrity of the gut in shrimp infected with WFS (Table 4 & Figure 5).

Treatment Group	**Hepatopancreas Lesion Score**	**Midgut Lesion Score**
Control	3.8 ± 0.4	3.5 ± 0.3
Probiotic	1.6 ± 0.3	1.4 ± 0.2
Prebiotic	1.9 ± 0.2	1.7 ± 0.3
Phytogenic	1.8 ± 0.2	1.6 ± 0.2
Immunostimulant Combo	1.3 ± 0.2	1.1 ± 0.1

Table 4. Histopathological Lesion Scores of Hepatopancreas and Midgut Tissues (0 = Normal, 5 = Severe Damage)

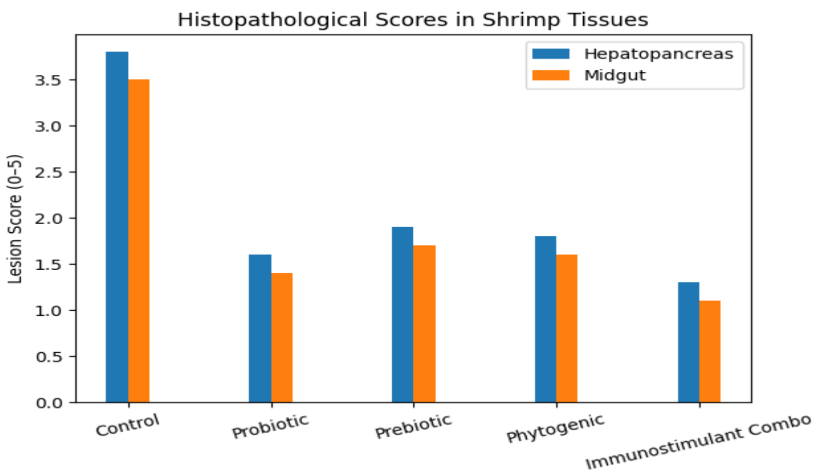

Figure 5: Histopathological Scores in Shrimp Tissues

Survival and WFS Incidence

The assessment of clinical effects after pathogen challenge showed that there were differences in survival and WFS incidence depending on the treatment. The immunostimulant-fed group had the highest cumulative survival rate (89.2 ± 2.1%) and the lowest WFS rate (10.4 ± 1.2%) and WFS mortality (3.5 ± 0.7%). On the other hand, the control group had the lowest survival rate of (68.5 ± 3.1%) and the highest WFS incidence rate of (41.2 ± 2.7%) and mortality rate of (21.6 ± 1.9%). The probiotic, prebiotic, and phytogenic treatments yielded moderate survival rates of between 84.1% and 86.4% and hence lower WFS rates. These outcomes depict the preventive effect of dietary supplementation on WFS, whereby the immunostimulant blend provided the highest disease resistance among the intervention measures (Table 5 & Figure 6).

Treatment Group	Cumulative Survival (%)	WFS Incidence (%)	Mortality Due to WFS (%)
Control	68.5 ± 3.1	41.2 ± 2.7	21.6 ± 1.9
Probiotic	86.4 ± 2.5	15.3 ± 1.8	5.7 ± 1.0
Prebiotic	84.1 ± 2.8	17.9 ± 1.5	6.8 ± 1.1
Phytogenic	85.3 ± 2.6	16.8 ± 1.6	6.2 ± 0.9
Immunostimulant Combo	89.2 ± 2.1	10.4 ± 1.2	3.5 ± 0.7

Table 5. Cumulative Survival Rate (%) and White Faeces Syndrome (WFS) Incidence After Experimental Challenge

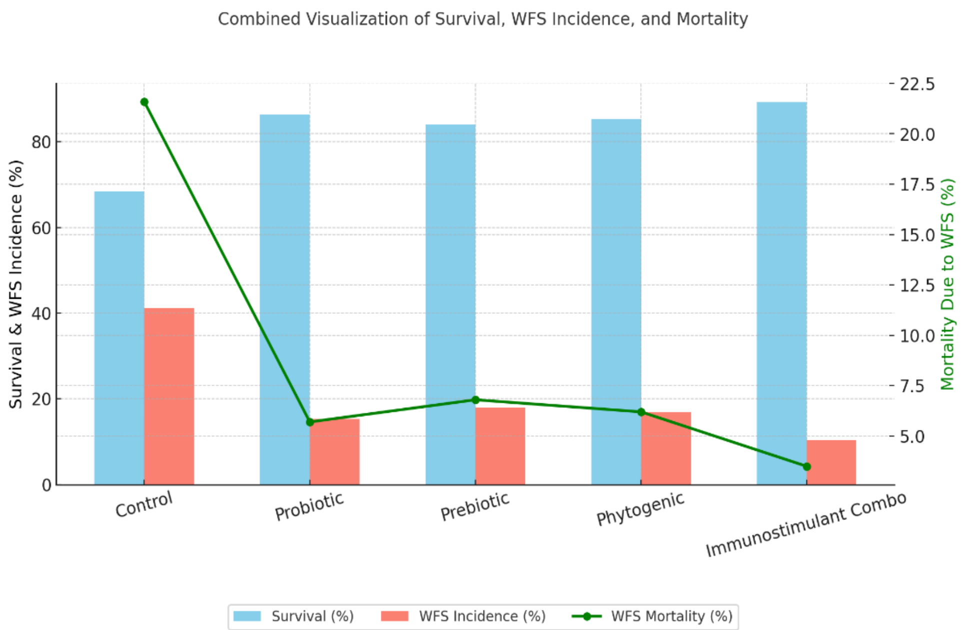

Figure 6: Combined visualization of survival, WFS Incidence, and Mortality

4. Discussion

The results of this study add to the existing literature that dietary supplementation is a feasible approach to addressing health issues in shrimp farming, especially WFS. It is considering these findings of enhanced growth performance among the supplemented groups, particularly immunotolerant that confirmed the nutritive and physiologic effectiveness of the functional feed additives. These findings are in conformity with other studies stressing the fact that immunostimulants like β-glucans and nucleotides improve feed conversion ratio and growth performance due to enhanced immune capacities and stress resistance in crustaceans (Bai *et al.*, 2014; El-Saadony *et al.*, 2022).

The increase in the innate immune markers in the current study supports the immunomodulatory effect of probiotics and phytogenic. Higher haematocrit value, total haemocyte count, phenol oxidase activity and antioxidant enzyme level in supplemented group further supports the previous study which shows that the interaction of the natural bioactive compounds enhances the immune system in the aquaculture fish (Akhter *et al.*, 2015). The observed enhancement in immunity, especially in the shrimp fed with the immunostimulant blend, would be due to enhanced receptor binding and activation of such pattern recognition that is important in the immune response against pathogens (Rahayu *et al.*, 2024).

The alteration of gut microbial distribution after supplement consumption, accompanied by the decrease in *Vibrio spp*. and the increase in remarkably beneficial bacteria like *Bacillus* and *Lactobacillus*, proves that the dietary approaches are capable to manage the intestinal microbiota. This microbial shift is important since dysbiosis has been linked to the development of WFS (Sathish Kumar *et al.*, 2022; Zakaria *et al.*, 2025). The increase in probiotic taxa not only increases colonization resistance but also helps in maintaining the stability of the intestinal barrier and inhibiting the activity of pathogenic factors.

The histopathological scores of supplemented shrimps also showed that the dietary additives have a protective role in reducing the tissue damage in the gut and hepatopancreas (Abd El-Naby *et al.*, 2024). In accordance with the other research, it implies that phytogenic and immunostimulants reduce histological lesions due to inflammation and oxidation (Citarasu, 2010; Idenyi *et al.*, 2022). The enhanced tissue morphology of the supplemented groups could have contributed to the ability to maintain physiological and digestive functions during pathogen exposure.

The most interesting is the fact that shrimp fed with immunostimulant-enriched diets have better survival and disease resistance. The lower WFS incidence and mortality in this group imply that the body has a strong systemic defense mechanism, which may involve phagocytosis and cytokine production (Eissa *et al.*, 2023). These findings support the role of functional feed ingredients in the prevention of diseases, which is a more realistic approach than the use of chemotherapeutic and antibiotic agents.

As this study reveals significant functional changes of the supplements incorporated in the diets, it also identifies the complicated nature of the affirmative interactions among the 3Ps of host, pathogen and environment. It is therefore possible that immunological priming, microbial modulation, and intestinal fortification are the factors that explain the observed health effects, and these should be investigated further using molecular and transcriptomics approaches. This integrated comprehension is important for developing the precise nutrition plans considering the specific type of the animal species and targeted condition.

This study proves that dietary supplementation is a very effective and sustainable method to prevent WFS in *Penaeus vannamei* using immunostimulants. All these interventions also enhance growth and immune status as well as microbial condition and tissue health. The use of such dietary solutions could be of great value for shrimp health management and future production as the aquaculture industry shifts towards antibiotic-free production.

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