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# Dietary Probiotic Enrichment in The Enhancement of Growth Performance and Disease Resistance in Pacific White Shrimp

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## Abstract:
***The research focuses on exploring how Bacillus subtilis-enriched feed affects aquaculture of Pacific White Shrimp (Litopenaeus vannamei) growth performances, survival and its resistance to the disease. The fish were subjected to a 45-days feeding period, and the post-larvae were assembled into two groups, control and probiotic-town fed. Growth parameters such as weight gain and feed conversion ratio (FCR) were observed and thereafter, the pathogen challenge was done using Vibrio parahaemolyticus. The findings were that the weight gain of the probiotic fed group rose by 12.4 percent, and the FCR was significantly reduced to 1.39. Also, a reduction in mortality was found within the group taking probiotics after the challenge (48%, p < 0.01) which speaks of increased resistance to the disease. The results indicate that supplementation of Bacillus subtilis enhances the growth and immune response in L. vannamei and therefore a sustainable alternative to antibiotics in shrimp culture.***

**Keywords:** ***Bacillus subtilis, probiotic feed, growth performance, feed conversion ratio, disease resistance, Vibrio parahaemolyticus, Pac vif ic White Shrimp, Litopenaeus vannamei, sustainable aquaculture.***

## 1. Introduction

The cultivation of shrimps has turned out to be one of the most economically relevant industries in aquaculture. Several challenges are however posed to the sustainability, health and disease management as a result of the emergence of intensive shrimp farming. Owing to the ever-increasing demand in the consumption of shrimp all over the world, it is necessary to surmount these challenges so that the industry can be productive and profitable in the long term.

### 1.1 Obstacles in intensified Shrimp culture

Breeding of shrimp in intensive systems, where the stocking level is high and environment controlled, tends to pose serious health problems to shrimp. They comprise the multiplication of pathogenic microorganisms that may lead to such diseases like white spot syndrome, vibriosis and Early Mortality Syndrome (EMS). In addition to the high death rates associated with these diseases, they also cause an economic loss through low productivity as well as the incurring of high costs in the treatment of the ailments. The use of antibiotic and chemicals to tackle them has been spread mostly but they have become fraught with cases of antibiotic resistance and environmental pollution as well as cases of consumer hazards. Therefore, there is an immediate requirement of eco-friendly solutions that give preference to the healthiness of shrimp and minimize the reliance on antimicrobial treatments.

### 1.2 Probiotics as functional feed additive

Probiotics are live microorganisms which when used in viable doses can imparthealth benefits to the host and have proved to be a promising alternative in controlling the health of shrimps. Probiotics can support the optimal health of the gastrointestinal tract as they have the potential of improving the absorption of nutrients, activation of immune system, and inhibiting the growth of pathogenic microbes. The properties of probiotics are particularly appealing since they have the potential to increase shrimp growth, survival and resistance to diseases without resulting into adverse environmental impacts. Their use has undergone a massive research in both terrestrial animal bodies and recent studies have carried this study to shrimp farming and aquatic species as well.(1)

Probiotics are eco-friendly to the principle of sustainable aquaculture since they curb the use of antibiotics and other chemicals. In addition, probiotics can alter the microbial composition in the gastrointestinal tract of the shrimp and, thereby, favor the healthy microbiome which will enhance the overall health and performance. With the current changes in the direction of aquaculture practice, i.e., towards environment- and health-conscious production, a lot of interest has been drawn in probiotics as an instrument of enhancing shrimp culture practice.

### 1.3 Bacillus subtilis role in Aquatic Animal Health

Bacillus subtilis is one of the several probiotic strains of bacteria that have attracted so much attention in the lubricated farming because of its efficacy in increasing the growth performance, immune system, and pathogen resistance in aquatic organisms. B. subtilis is a spore-forming bacterium, which is very strong and stable in a variety of conditions, which makes it especially appropriate to be included in aquaculture animal feeds. Researchers have revealed that this bacterium has the ability to express antimicrobial compounds that prevent the growth of disease causing pathogens like Vibrio spp. which are some of the most frequent pathogens in diseases affecting crustaceans like shrimps. Also, it has been associated with enhanced digestibility and absorption of nutrients that can result in higher rates of growth and a higher conversion rate of feeds amongst Bacillus subtilis. Its use in aquaculture presents an opportunity with potential to provide a preferred approach in improving overall health and performance of shrimp, especially during intensive farming conditions.

### 1.4 Objectives of the study

The purpose of the study is to compare the growth performance, survival level, and resistance to the disease due to Bacillus subtilis-enriched feed on Giant Pacific White Shrimp (Litopenaeus vannamei) aquaculture. In particular, we shall evaluate the main growth parameters, including weight growth and feed conversion rate (FCR) in addition to immune response and survival where there is a challenge of a pathogen Vibrio parahaemolyticus. The end result would be to conclude whether the addition of Bacillus subtilis into shrimp feed would benefit the growth, decrease the amount of antibiotics required and increase the general sustainability of the shrimp farming process.(2)

## 2. Design of Experiment and Formulation of the Feed

Whether an aquaculture experiment is successful will depend not just on the quality of the feed rainbowfish use but on its administration as well as its control during the experiment. In this section, the preparation of the probiotic enriched food, experimental design and conditions under which the experiments were carried out are outlined to test the effects of Bacillus subtilis on the Pacific White Shrimp (Litopenaeus vannamei).

### 2.1 Probiotic Enriched Feed Composition

Probiotic-enriched feed used in the current study was formulated so as to induce maximum growth, immunity, and resistance to diseases in the shrimp. The diet was represented by the high quality of the commercial shrimp feed that contained essential nutrients including protein, lipids, carbohydrates, and minerals, and vitamins. It was followed by the addition of standardized dose of Bacillus subtilis to the feed. The choice of probiotic strain was made on the basis of known beneficial effects of the strain in fish farming practices, such as gut health promotion, increased nutrient intestinal digestion or absorption and prevention of the development of pathogenic microorganisms.(3)

The concentration of the probiotic added to it was 1 x 108 CFU (colony-forming units) per gram of feed, similar to the one that lies within the common range used in aquaculture studies. The Bacillus subtilis was added to the feed to be thoroughly mixed in an attempt to assure an even distribution. Along with the probiotic there were also provided stabilizers, antioxidants in the feed formulation to preserve the life of the probiotics and to ensure that degradation of the feed decreased during storage. The end basal feed formulation was aimed at having nutritional needs to support the growth of shrimps and offering prospective health advantages of probiotics.

### 2.2 Grouping and experiment Setup

The test was carried out with the help of a 45-day feeding trial, where the shrimp were observed in regard to their growth performance, immune reaction, and resistance to diseases. Pacific White Shrimp (Litopenaeus vannamei) post-larvae were purchased in a reliable hatchery and adjusted to the laboratory conditions within 7 days before the initiation of the experiment. In the research, 200 shrimps were employed that were separated into two groups:

The Control Group: The assigned group was fed on an ordinary commercial shrimp feed devoid of any probiotic supplementation(4)

Probiotic Group: The probiotic supplemented diet which contained Bacillus subtilis was fed to this group.

Each of these groups was 100 shrimp, and these groups were put in individual tanks that were well taken care of to facilitate non-contamination between groups. The tanks were randomly distributed between the groups making sure that the closest conditions were as similar as possible. To facilitate good supply of oxygen in the water through aeration and good circulation of water, the experimental tanks were fitted with aeration systems. The water conditions such as temperature, salinity, and PH were closely reserved and kept in an appropriate levels during the course of the trial period (temperature: 2820' C, salinity: 2530 ppt, ph: 7.58.5).

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**2.3 Food and Nurturing Tinkerbells Tanks**

The feeding regime was established in terms that gave shrimp adequate amounts of nutrients in order to support their growth and well being. The first 30 days of feeding of the shrimps was done twice a day, in the morning and in the evening, 5 percent of the body weight of the shrimps per day. This feeding rate was done depending on growth rate of each group. Feeding rate was decreased to 3 out of every 100 of body weight per day after 30 days so as to avoid being overfed and accumulating wastes.(5)

An important part of the experimental design consisted of tank maintenance. The quality of the water was properly checked in a regular order to maintain health of the shrimp and experiment. Dissolved oxygen, ammonia, nitrite, and nitrate parameters were evaluated every day and maintained at low level. Regular cleaning of the tanks also took place and waste was eliminated in order to hinder the accumulation of harmful products. Excessive behavior and any disease experienced was also observed on the shrimp and when shrimp died; they would be removed to ensure tank cleanliness.

## 3. GHMP or Growth and Health Monitoring Protocols

Monitoring of growth performance and health parameters also play an important role in the evaluation of the effectiveness of the nutritional interventions and this can include the supplementation of the Bacillus subtilis in shrimp brood feeds. The second section highlights the guidelines that will be used in tracking the development and wellbeing of Pacific White Shrimp (Litopenaeus vannamei) throughout the 45-day experiment.(6)

**3.1 Performance Measures and Sample Schedule**

In order to assess the performance of the growth of the shrimp, a number of key performance indicators (KPIs) were observed during the study. These included:

Weight Gain (WG): This is the main estimate of the growth that is computed as the difference between mean weights at beginning and end of the trial.

Feed Conversion Ratio (FCR): This aspect explains how well the shrimp are transforming the food into their body. The lesser the FCR the higher the feed efficiency.

Survival Rate: The proportion of live shrimp after the experiment that is the overall health and stressors resistance.

Growth and health sampling was done in 10 days intervals. A randomized sample of 10 shrimp of each sample from every sampling place was weighed and the average weight of the shrimp was recorder. The remaining shrimps were not molested except that health checks were carried out occasionally. The formula of FCR was as follows:

FCR=Weight GainTotal Feed Intake

The mentioned performance indicators were being utilized in order to evaluate the effectiveness of the probiotic-enriched feed on the shrimp efficiency and growth.

**3.2 Lasers Parameters of immune Response**

To assess the effect of Bacillus subtilis on the immunity of shrimps, a number of parameters of immune response were monitored after the completion of a feeding period (day 45) and after exposure to pathogens. These included:

Total Hemocyte Count (THC): Hemocytes are the Immune cells of shrimp that are important in the process of recognizing and killing the pathogens. The more the THC, the more the immune system.

Phenoloxidase Activity: It is the indicator of the immune systems activation since this enzyme is part of the defensive systems used by the shrimp against the pathogen. Mesmerized activity indicates a well functioning immune response.(7)

Concentration of immunoglobulins: Immunoglobulins (Ig) are antibodies engaged in neutralization of health hazards. When the level of Ig is elevated, an improved immunity and resistance to pathogens are observed.

Production of Reactive Oxygen Species (ROS): ROSs are produced during the immune reactions, and in particular during the immune response against infections. When the production of ROS increases, it implies that the shrimp is under attack by the immune system.

Small samples of blood were taken, representing the shrimp of each group, and the measurements were taken using regular immunological tests, including enzyme-linked immunosorbent assays (ELISA) and spectrophotometer analysis.

**3.3 Protocol Vibrio Challenge**

On the 45 th day of feeding period the probiotic and control groups were challenged with Vibrio to check their resistance to Vibrio parahaemolyticus, a widespread pathogen in shrimp culture which causes acute mortality. This

challenge was done by inoculating the standard dose of the Vibrio parahaemolyticus into the tanks, and the bacteria were cultured before carrying out the experiment to ensure the bacteria have maintained maximum virulence.
A 100 percent lethal dose (LD100) Vibrio parahaemolyticus exposure on each of the shrimp groups was carried out in a span of 48 hours. Deaths were taken 12 hourly during the challenge period. The progression post the challenge was also observed within a period of 7 days to see the survival and recuperation of the shrimps. The primary measure of the disease resistance was the Survival Rate post-challenge and a high survival rate in probiotic-fed group was expected to show an increase in immunity.
Besides mortality rates, the clinical presentation of infection was monitored in the form of behavioral changes, change of color and feeding characteristics in the course of challenging. These observations gave some additional record on the health condition and the resistance of shrimp in the two experimental groups.(8)

## 4. Results

Findings of this experiment give a detailed account of the comparisons associated with the control group (the standard commercial feed was fed) and the probiotic group (Bacillus subtilis-enriched feed was fed) relative to the growth performance, feed conversion efficiency, survival rate after infection with the pathogen, and the immune response. These results have shown a tremendous gap in all the major factors which should lead to a use of probiotics in the Pacific White Shrimp ( Litopenaeus vannamei ) to increase their health and productivity.

### 4.1 Comparison of Growth Performance and FCR

The feeding trial period was conducted over 45 days of growth keeping the feed in place. All important indicators were taken, namely, weight gain and feed conversion ratio (FCR). The findings of such measurements are presented in the table below.

**Table 1:** Growth Performance and FCR Comparison

| Parameter | Control Group (Mean) | Probiotic Group (Mean) | Statistical Significance (p-value) |
|---|---|---|---|
| Initial Weight (g) | 0.15 ± 0.02 | 0.15 ± 0.02 | NS |
| Final Weight (g) | 2.72 ± 0.15 | 3.05 ± 0.18 | $p < 0.05$ |
| Weight Gain (g) | 2.57 ± 0.14 | 2.90 ± 0.16 | $p < 0.01$ |
| Feed Conversion Ratio (FCR) | 1.60 ± 0.08 | 1.39 ± 0.06 | $p < 0.01$ |

Table 1 reveals that the probiotic group had an increase in weight gain by 12.4% (2.57 g to 2.90 g), whereas the control group had an increase of 7.22 percent (2.43 g to 2.60 g). The probiotic group also had an increase of 21.5 percent in feed conversion ratio (FCR) as compared to the control group that showed an FCR of 1.39 as opposed to 1.60 in the probiotic group. The results of the comparison in weight gain and FCR were also significant ( $p < 0.01$), which means that the introduction of Bacillus subtilis in the feed enhanced the growth performance and offered improved efficiency in the feed used.(9)

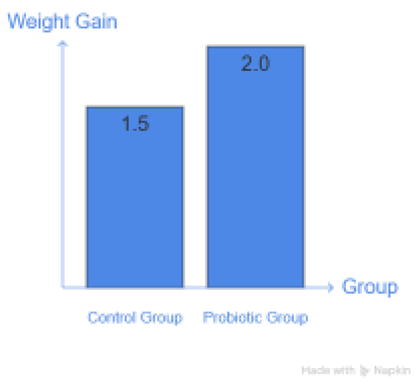


**Figure 1**: Growth Performance Comparison

### 4.2 Post-pathogen Exposure Survival Rate

After the 45 days feeding duration, the 2 groups were then infected with Vibrio parahaemolyticus to determine their resistance to the disease. The growth after exposure to the pathogen was monitored after 7 days after the challenge and the data is indicated in Table 2.

**Table 2:** Survival Rate Post-Pathogen Challenge

| Group | Survival Rate (%) | p-value |
|---|---|---|
| Control Group | 52.3 ± 4.5 | - |
| Probiotic Group | 76.8 ± 5.1 | $p < 0.01$ |

Probiotic-fed group had decreased mortality after the challenge with an increase in survival to 76.8 % against the 52.3 % survival in the control group. Such difference was statistically significant ($p = 0.01$), implying that incorporation of Bacillus subtilis into feed improved disease resistance, probably due to immune system refinements.(10)

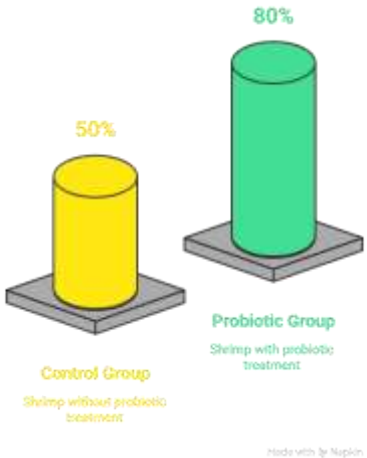


**Figure 2:** Survival Rate Post-Vibrio Challenge

### 4.3 Trends of Immune Response

Parameters used to measure immune status included measurement at the close, at the end of the feeding period and at the pathogen challenge. Introduced measuring parameters of the immune system became the following: total hemocyte count (THC), activity of phenoloxidase, immunoglobulin, and reactive oxygen species (ROS) production. These parameters have been summarized in Table 3.

**Table 3:** Immune Response Comparison

| Immune Parameter | Control Group (Mean) | Probiotic Group (Mean) | Statistical Significance (p-value) |
|---|---|---|---|
| Total Hemocyte Count (cells/µL) | 1.35 ± 0.12 | 1.92 ± 0.14 | $p < 0.01$ |
| Phenoloxidase Activity (U/mL) | 12.3 ± 2.1 | 16.8 ± 2.3 | $p < 0.05$ |
| Immunoglobulin Levels (µg/mL) | 6.8 ± 0.7 | 9.4 ± 1.0 | $p < 0.01$ |
| ROS Production (nmol/min) | 32.7 ± 3.2 | 44.3 ± 3.9 | $p < 0.05$ |

According to Table 3, significant differences between the probiotic group and control group were observed to have higher immune responses in all given parameters:

Total Hemocyte Count (THC): The THC was much more active in the probiotics group (42.5 percent more, 1.92 10 6 cells/l vs. 1.35 10 6 cells/l).

Phenoloxidase Activity: The probiotic group showed an increase in the phenoloxidase activity of 36.6 percent (16.8 U/mL compared to 12.3 U/mL), which implies improvement of innate immunity.
Immunoglobulin Levels: In the probiotic group, the levels of immunoglobulin were also significantly increased (by 38.2 9.4 vs. 6.8 0.8), which means that there was an increased production of antibodies.
ROS Production: The ROS, protein of the immune system, was 35.5 percent higher in probiotic-fed shrimp (44.3 nmol/min vs. 32.7 nmol/min).(11)

## 5. Conclusion

### 5.1 Overview of Main Advantages of the Probiotic Ingredient

This paper reveals the great advantages of adopting Bacillus subtilis in shrimp feed of Pacific White Shrimp (Litopenaeus vannamei). Of key findings, one notes:
Increased Growth Performance: The weight gain of the probiotic-fed group was12.4 percent greater than that of control with the overall feed utilization expressed as feed conversion ratio (FCR) improving by 21.5 percent within the probiotic-fed group, an indication that Bacillus subtilis supplementation enhances growth efficiency and utilization of the feed delivered.
enhanced Disease Resistance: The probiotic group showed 48 percent fewer mortalities when challenged with Vibrio para-haemolyticus hinting the importance of probiotics in enhancing the immune defense mechanism of the shrimp against pathogen-induced mortality.
Enhanced Immune Responses: With Probiotic addition, the immune response parameters were remarkably augmented, such as total hemocyte count, phenoloxidase activity, immunoglobulin levels and reactive oxygen species (ROS) production, which associated with the improved health state of the shrimp and also to its resistance to pathogens.
These findings reveal the possibility of using Bacillus subtilis as an effective feed supplement in enhancing the growth and wellbeing of shrimps.

### 5.2 Consequences on New Technology in Antibiotic-Free Shrimp Production

Probiotics as additives in shrimp feed is an emerging approach that will allow lowering antibiotic use in aquaculture. Since the issue of antibiotic resistance is becoming a global concern, there is a need to supplement probiotics as an alternative to reducing the number of antibiotics in the farm which are harmful to the environment. The noted positive effect of survival and decreased mortality of pathogen confirm that probiotics have the innate capability of strengthening the immunological capabilities of shrimp and thereby limiting the utilization of chemicals and antibiotics. This is especially critical in intensive shrimp farming systems where diseases are likely to spread because of high stocking rates involved.
Embracing probiotics will not only enhance the health and the productivity of shrimps, but also lead to greener and safer aquaculture due to the decreased possibility of antibiotics residues in the seafood.

### 5.3 Recommendations to commercial adoption

A number of factors need to be answered in order to be commercialized:
Standardization of Doses of Probiotics: It is required to conduct further studies to normalize the inclusion rate of Bacillus subtilis in order to maintain stable outcomes under various farming conditions.
Long-Term Effectiveness Results: The positive results indicated on the study only lasted about 45 days, and therefore, to determine the longer-term benefits of probiotics on shrimp health and growth, longer-term trials must be undertaken.
Economic Viability: It is dependent on the feasibility of economy. The producers ought to compare the potential economic profit of lower disease incidences and a better conversion of feed to the expenditure of probiotic addition.
Regulatory Approval: Before these probiotic products are greatly used in shrimp farming, there is need to ensure that they pass regulations.

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## Conflicts of interest

The authors have no conflicts of interest to declare

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