



Contents lists available at openscie.com

E-ISSN: 2776-7205

Applied Research in Science and Technology

DOI: 10.33292/areste.v5i1.89

Journal homepage: <https://areste.org/index.php/oai>



The Effectiveness of Adding Immunostimulants, Probiotics, and Liming Methods on Water Quality and Productivity of Shrimp *Litopenaeus vannamei* in Dewi Laut Aquaculture Company, Garut, Indonesia

Sheny Permatasari^{1*}, Amalia Putri Firdausi¹, Yassar Ariq Pradian¹, Ivana Oktarina Sopacua²

¹ Technology and Management of Applied Aquaculture, School of Vocational Studies, IPB University, Bogor, Indonesia

² PT. Dewi Laut Aquaculture, Garut, West Java, Indonesia

*Correspondence: E-mail: shenypermata@apps.ipb.ac.id

ARTICLE INFO

Article History:

Received 06 June 2025

Revised 22 June 2025

Accepted 24 June 2025

Published 28 June 2025

Keywords:

Immunostimulants,

Lime,

Litopenaeus vannamei,

Probiotics,

Water Quality.

ABSTRACT

Background: The whiteleg shrimp *Litopenaeus vannamei* is one of Indonesia's leading commodities. However, intensive culture of *Litopenaeus vannamei* faces challenges such as environmental stress and decreased productivity due to fluctuating water quality.

Aims & Methods: This study aimed to evaluate the effectiveness of adding immunostimulants (vitamin-mineral mix and garlic extract at 2 ppm), probiotics (2 ppm every two weeks), and lime treatment (CaO at 10 ppm) on water quality and the productivity of *L. vannamei* in ponds at Dewi Laut Aquaculture Company, Garut. The observed productivity parameters included Average Body Weight (ABW), population, biomass, Feed Conversion Ratio (FCR), Survival Rate (SR), and size. Meanwhile, the measured water quality parameters included physical parameter such as temperature, chemical parameters such as pH, dissolved oxygen (DO), total ammonia nitrogen (TAN), total alkalinity, and total organic matter (TOM), as well as biological parameters such as total phytoplankton, total bacteria count (TBC), and total vibrio count (TVC).

Result: The results showed that the applied treatments produced good culture performance, with ABW reaching 13.37 grams, SR at 91.01%, FCR at 1.7 and productivity of 20.39 tons/ha. Meanwhile, water quality monitoring indicated that some parameters exceeded the ideal ranges for shrimp culture, such as pH (maximum 9.12), TOM (up to 134 ppm), and TAN (up to 2.6 ppm). However, there is synergy between probiotics and immunostimulants, these conditions were still tolerable for the shrimp.

To cite this article: (2025). The effectiveness of adding immunostimulants, probiotics, and liming methods on water quality and productivity of shrimp (*Litopenaeus vannamei*) in Dewi Laut Aquaculture Company, Garut, Indonesia. *Applied Research in Science and Technology*, 5(1), 97-107.

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1. Introduction

To address these issues, various environmentally friendly innovations have been developed, including the use of immunostimulants, probiotics, and liming methods to enhance shrimp growth performance and maintain a stable culture environment. Immunostimulants such as vitamins, minerals, and natural herbal compounds have been shown to improve the non-specific immune responses in shrimp. Pazir *et al.*, (2017) reported that dietary supplementation with 2% garlic extract significantly enhanced growth and immune function in *L. vannamei*. Garlic also exhibits antimicrobial properties that can benefit the culture environment (Delgado *et al.*, 2023).

In addition to immunostimulants, probiotics are widely used to balance intestinal microflora and improve water quality. Research by Anwar *et al.*, (2016) demonstrated that supplementation of commercial probiotics (*Lactobacillus plantarum*, *Bacillus* spp., *Bacillus subtilis*, *Nitrosomonas europea*, *Bacillus licheniformis*, *Nitrosomonas* spp., *Nitrobacter* spp., *Lactobacillus* spp., and *Geobacillus* spp.) in shrimp feed significantly affected growth rate and feed efficiency in *L. vannamei*. Similarly, Dewi *et al.*, (2023) found that the administration of *Lactobacillus* spp. and *Bacillus* spp. at varying dosages significantly improved both absolute weight gain and daily growth rate in *L. vannamei*.

To maintain overall water quality, pond liming using quicklime (CaO) is a common practice aimed at stabilizing pH, suppressing pathogenic microorganisms, and accelerating the decomposition of organic matter. Yunus *et al.*, (2020) reported that the application of quicklime and dolomite increased molting frequency, absolute weight gain, and daily growth rate in *L. vannamei*. Moreover, various types of lime-such as dolomite, calcite, and quicklime-have been shown to significantly increase water pH, with quicklime demonstrating superior phosphorus solubility compared to other types (Febriani *et al.*, 2024). The reaction between quicklime and water produces calcium hydroxide, which elevates water pH and helps control pests and pathogens by creating an unfavorable environment for harmful organisms (Rahman & Abdullah, 2020).

Given this background, the combined use of immunostimulants (vitamins, minerals, garlic extract), probiotics, and liming presents a promising strategy to improve *L. vannamei* culture performance. Therefore, this study aims to evaluate the effectiveness of this combination in enhancing water quality and shrimp productivity in the intensive ponds of Dewi Laut Aquaculture Company, Garut, Indonesia.

2. Methods

2.1 Study Site and Duration

The shrimp (*Litopenaeus vannamei*) culture experiment was conducted at Dewi Laut Aquaculture Company, located at Jl. Raya Lintas Selatan, Cigadog Village, Cikelet District, Garut Regency, West Java Province, Indonesia (44177). The research was carried out from June 22 to September 4, 2023.

2.2 Pond Preparation

The shrimp were cultured in High Density Polyethylene (HDPE)-lined ponds with an coverage area of 400 m². Pond preparation began with cleaning using water from a sterilization pond, followed by a 14-day drying period aimed to eliminate pathogenic organisms and evaporate harmful organic residues. During the drying period, paddlewheel aerators were cleaned and tested for proper function. The water source was nearby seawater, which was pumped in, filtered through coral filters, and sterilized using Trichloroisocyanuric acid (TCCA). The sterilized water was then aerated using paddlewheels for 24 hours and supplemented with minerals such as calcium, magnesium, sodium, and soluble potash before being introduced into the ponds.

2.3 Test Animal Preparation

The experimental animals were *L. vannamei* post-larvae (PL 9–10), classified as F1 generation and certified as Specific Pathogen Free (SPF) to ensure quality. The post-larvae were sourced from CP

Pangandaran. An intensive stocking density of 160 individuals/m² was applied. Prior to stocking, the shrimp were acclimatized to the pond's temperature and salinity conditions.

2.4 Application of Immunostimulants and Probiotics in Feed

This study employed a descriptive approach to evaluate the application of immunostimulants and probiotics in shrimp feed, along with the liming method used in *L. vannamei* cultivation at Dewi Laut Aquaculture Company, Garut.

Vitamins and minerals (using the commercial products Imun-CE contains vitamin C, E, and selenium; and Vitalar contains microminerals and beta glucan) and garlic extract (Alisin brand) were administered orally through a feed-coating method. All substances were dissolved in water at a concentration of 2 ppm and used to coat the feed, which was left to stand for two hours before feeding.

The probiotic Biomin (contains *Bacillus subtilis*, *Bacillus licheniformis*, etc) was applied at a dose of 0.5 kg/ha every two weeks. Prior to application, probiotic Biomin was soaked in seawater for two hours and then distributed in the afternoon to promote microbial balance in the pond ecosystem.

2.5 Liming Method

Liming was conducted by soaking quicklime (CaO) at a dosage of 10 ppm for 12 hours. The quicklime was mixed with water in a container and stirred twice: initially during mixing and again just before application. The quicklime solution was distributed into the ponds through a PVC pipe system connected to a valve at the bottom of the container.

2.6 Test Animal Maintenance

Feeding

Feeding affects the growth and development of *L. vannamei*. The feeding methods used are the blind feeding and demand feeding methods. The feeding program can be seen in Table 1.

Table 1. The feeding program of *L. vannamei*

| DOC | Frequency | Feeding Time | Feeding Method |
|------------|---------------|--|----------------|
| 1-30 | 4 Times a day | 06.00 WIB, 10.00 WIB, 14.00 WIB, 18.00 WIB | Blind Feeding |
| 31-harvest | 4 Times a day | 06.00 WIB, 10.00 WIB, 14.00 WIB, 18.00 WIB | Demand Feeding |

The blind feeding method in this study was carried out with the assumption that every 100.000 fry were given 3 kg of feed, adjustments based on population and growth targets. [Ritonga \(2021\)](#) states that the provision of *L. vannamei* feed is calculated with the assumption that 100.000 fry are given 1-3 kg of feed.

The demand feeding method start from DOC 31 until harvest. This method is carried out by checking the anco as an indicator of feed reduction. If in one feeding time there is remaining feed in the anco, then in the next feeding time the feed will be reduced.

Growth Monitoring

The growth of *L. vannamei* was monitored through regular sampling to determine the average body weight (ABW). Sampling was conducted using feeding trays (anco) and nets. The growth monitoring schedule is presented in Table 2.

Table 2. The growth monitoring program of *L. vannamei*

| DOC | Sampling Time | Analysis | Sample |
|------------|---|---|--------|
| 1-30 | Every 10 days | Measurement of biomass and average weight | >30 |
| 31-35 | Every 7 Days | Measurement of biomass and average weight | >30 |
| 36-harvest | Every 7 days and one day before harvest | Measurement of biomass and average weight | >30 |

2.7 Measured Parameters

The parameters measured in this study included *L. vannamei* productivity and water quality. Productivity parameters consisted of average body weight (ABW), survival rate (SR), feed conversion ratio (FCR), and overall productivity. Water quality parameters included physical (temperature), chemical (pH, dissolved oxygen [DO], total ammonia nitrogen [TAN], total alkalinity, and total organic matter [TOM]), and biological components (total bacterial count [TBC], total vibrio count [TVC], and total phytoplankton abundance).

2.8 Growth Parameters

Average Body Weight (ABW)

ABW refers to the mean body weight of shrimp, measured in grams (g). This parameter represents individual growth over the culture period. The formula used refers to [Wahyudi et al. \(2022\)](#):

$$ABW(g) = \frac{\text{Shrimp weighing scale (gram)}}{\text{Number of shrimp (tails)}}$$

Survival Rate (SR)

SR indicates the percentage of shrimp that survived during the culture period relative to the number of post-larvae stocked. This is a critical parameter for assessing survival in aquaculture systems. The formula used refers to [Wahyudi et al. \(2022\)](#):

$$SR(\%) = \frac{\text{Number of live shrimp}}{\text{Number of shrimps spread}} \times 100\%$$

Feed Conversion Ratio (FCR)

FCR measures the efficiency of feed utilization in increasing shrimp biomass. Lower FCR values indicate better feed efficiency. The formula used refers to [Pratama et al. \(2017\)](#):

$$FCR = \frac{\text{Total feed given (g)}}{\text{Total biomass gain (g)}}$$

Productivity

Productivity refers to the total shrimp biomass produced per unit area over the culture period, typically expressed in tons per hectare (ton/ha). The formula used refers to [Wahyudi et al. \(2022\)](#):

$$\text{Productivity} = \frac{\text{Total biomass (kg)}}{\text{Pond area (ha)}}$$

Biomass

Biomass represents the total weight of the shrimp population at a given time. The formula used refers to [Effendi \(2000\)](#):

$$\text{Biomass} = \text{Population (tails)} \times \text{ABW (g)}$$

Population

Population indicates the total number of shrimp in a given period, based on the total biomass and ABW. The formula used refers to [Effendi \(2000\)](#):

$$\text{Population} = \frac{\text{Total Biomass}}{\text{ABW}}$$

Size

Size refers to the number of shrimp per kilogram. The formula used refers to [Nisa et al. \(2024\)](#):

$$\text{Size} = \frac{1000 \text{ g}}{\text{ABW}}$$

2.9 Water Quality Parameters

Water quality assessment was conducted using several key parameters. pH was measured twice daily (morning and afternoon) at a depth of 30 cm using a calibrated pH meter (EcoSense pH 100A) with standard buffer solutions. Dissolved oxygen (DO) and temperature were measured three times daily at the pond center using a DO meter (YSI Pro20).

Total alkalinity was measured by titration using 0.02 N sulfuric acid (H₂SO₄) and phenolphthalein as well as bromocresol green-methyl red indicators. Total organic matter (TOM) was measured through titration with potassium permanganate (KMnO₄) following preheating and reaction with oxalic acid. Total ammonia nitrogen (TAN) was measured spectrophotometrically using phenol, sodium nitroprusside, and an oxidizing reagent. Absorbance was recorded at 640 nm, and TAN concentration was determined using a standard calibration curve.

Total bacterial count (TBC) and Total vibrio vount (TVC) in the water were determined using the spread plate method on TSA and TCBS agar media, respectively. Plates were incubated at 35°C for 24 hours before colony enumeration. Plankton density was quantified using a hemocytometer based on cell counts from water samples collected with a dark bottle sampler.

2.10 Data Analysis

The collected data were tabulated and analyzed descriptively. Descriptive analysis involve summarizing or interpreting the collected data in a meaningful way to describe patterns or trends ([Sugiyono, 2015](#)).

3. Results

3.1 Growth Performance and Survival Rate

The results of observations on the average body weight (ABW), survival rate (SR), and productivity of *L. vannamei* over a 91-day culture period at the Dewi Laut Aquaculture Company farm in Garut are presented in Table 3.

Table 3. Growth performance, survival rate, and productivity of *L. vannamei* during the 91-day culture period.

| No | Parameters | Result |
|----|-------------------------|--------|
| 1 | DOC (days) | 91 |
| 2 | ABW (gram) | 13.37 |
| 3 | Size (individuals/Kg) | 74.85 |
| 4 | Biomass (kg) | 815.34 |
| 5 | Population (individual) | 60.93 |
| 6 | SR (%) | 91.01 |
| 7 | FCR | 1.7 |
| 8 | Productivity (ton/ha) | 20.39 |

The test pond demonstrated excellent production performance, indicating a successful intensive culture of *L. vannamei*. Feed efficiency was reflected by a feed conversion ratio (fcr) of 1.7 which is within the optimal range for intensive shrimp farming. The pond productivity reached 20.39 tons/ha, indicating high yield and efficient land use.

3.2 Water Quality Parameters

3.2.1 Physical and Chemical Parameter

The results of physical and chemical water quality measurements taken during the culture period are presented in Table 4.

Table 4. Physical and chemical water quality parameters during *L. vannamei* cultivation

| Parameters | Measurement | | | PerMen KP Nomor 75/Permen-Kp/2016 |
|------------------------------|-------------|-------------|-------------|--------------------------------------|
| | Morning | Afternoon | Night | |
| Temperature (°C) | 24 - 28 | 25 - 30 | 24 - 29 | 28-32 |
| pH | 7.21 - 8.81 | 7.49 - 9.12 | - | 7.5 - 8.5 |
| DO (ppm) | 4.02 - 5.90 | 5.49 - 9.10 | 3.36 - 5.11 | >3.0 |
| Total Organic Matter (ppm) | 80 - 134 | - | - | <90 |
| Total Alkalinity (ppm) | 150 - 269 | - | - | 100-250 |
| Total Ammonia Nitrogen (ppm) | 0 - 2.6 | - | - | <0.01 |

Water temperature ranged from 24–28°C in the morning, 25–30°C in the afternoon, and 24–29°C at night. pH values were recorded between 7.21–8.81 in the morning and 7.49–9.12 in the afternoon. Dissolved oxygen (DO) levels ranged from 4.02–5.90 ppm in the morning, 5.49–9.10 ppm in the afternoon, and 3.36–5.11 ppm at night. Total organic matter (TOM) levels ranged from 80 to 134 ppm, while total alkalinity ranged from 150 to 269 ppm. Total ammonia nitrogen (TAN) concentrations were recorded between 0 and 2.6 ppm.

3.2.2 Biological Parameter

Total Phytoplankton

One of the key biological parameters measured was total phytoplankton abundance. The results of total phytoplankton throughout the culture period are presented in Figure 1.

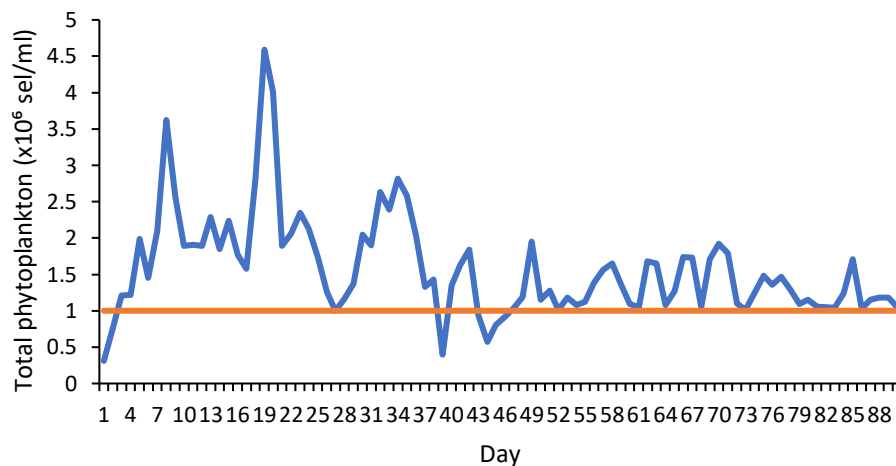


Figure 1. Total phytoplankton abundance in the *L. vannamei* culture pond during one production cycle.

Phytoplankton density exhibited dynamic fluctuations throughout the culture period of *L. vannamei*. On day 1, phytoplankton levels were low, followed by a sharp increase reaching a peak around day 18 at approximately 4.8×10^5 cells/mL. After the peak, the population declined and continued to fluctuate until the end of the cycle (day 88). Toward the end, the density stabilized below the maximum threshold level, around 1.5×10^5 cells/mL.

Total Bacteria Count (TBC) and Total Vibrio Count (TVC)

TBC and TVC were measured weekly over 13 weeks during the shrimp grow-out period, and the results are shown in Table 5.

Table 5. Weekly measurements and percentage composition of TBC and TVC during *L. vannamei* culture.

| Day | Total Bacteria (cfu/ml) | Total Vibrio(cfu/ml) | Percentage TV/TB (%) |
|---------|-------------------------|----------------------|----------------------|
| 1 | 1.2x10 ⁴ | 2.1x10 ³ | 16 |
| 2 | 2.7x10 ⁴ | 2.4x10 ³ | 9 |
| 3 | 3.2x10 ⁴ | 3.0x10 ³ | 10 |
| 4 | 1.8x10 ⁵ | 1.5x10 ⁴ | 11 |
| 5 | 2.0x10 ⁵ | 6.0x10 ³ | 5 |
| 6 | 5.3x10 ⁵ | 7.3x10 ³ | 2 |
| 7 | 7.9x10 ⁵ | 1.2x10 ⁴ | 2 |
| 8 | 3.5x10 ⁵ | 1.0x10 ⁴ | 3 |
| 9 | 1.9x10 ⁵ | 6.7x10 ³ | 5 |
| 10 | 2.1x10 ⁵ | 7.3x10 ³ | 8 |
| 11 | 2.0x10 ⁵ | 1.3x10 ⁴ | 9 |
| 12 | 2.6x10 ⁵ | 3.9x10 ⁴ | 25 |
| 13 | 1.7x10 ⁵ | 2.0x10 ⁴ | 12 |
| Average | 2.4x10 ⁵ | 1.1x10 ⁴ | 9 |

Total bacterial counts ranged from 1.2×10⁴ to 7.9×10⁵ colony-forming units per milliliter (cfu/mL), with an average of 2.4×10⁵ cfu/mL. The highest bacterial count was recorded in week 7, while the lowest was observed in week 1.

The total vibrio count ranged from 2.1×10³ to 2.0×10⁴ cfu/mL, with an average of 1.1×10⁴ cfu/mL. The proportion of *Vibrio* relative to total bacteria (TV/TB) ranged from 2% to 25%, averaging 9%. The highest proportion of *Vibrio* occurred in week 12, while the lowest percentages were recorded in weeks 6 and 7.

4. Discussion

4.1 Shrimp Productivity

The results of this study on the productivity parameters of *L. vannamei* indicate that the combination of immunostimulants, probiotics, and liming methods had a positive effect on the survival rate (SR) of *L. vannamei*. The observed SR of 91.01% was considered very high, exceeding the common range of 80–85% in intensive farming systems (Witoko *et al.*, 2018). This improvement was supported by the administration of commercial garlic extract (Alisin) and a vitamin supplement (Imun-CE). Alisin acts as a natural antibiotic and contains attractant compounds that enhance shrimp appetite, while Imun-CE contains vitamin C, vitamin E, and selenium, which play crucial roles in boosting immune response and promoting growth (Marani 2014). Vitamin C, in particular, is known to enhance stress resistance, immune function, and molting recovery. The efficacy of garlic extract in improving survival was also demonstrated by Javadzadeh *et al.*, (2012), who reported optimal growth and survival in *L. vannamei* larvae fed *Artemia* enriched with 200 mg/kg garlic extract. The active compound allicin functions as a natural antibacterial and immunostimulant (Miron *et al.*, 2000), and has been shown to stimulate the immune system and enhance disease resistance in aquatic organisms, partly by affected hemocyte counts and total plasma protein (Samadi *et al.*, 2016). Higher hemocyte levels indicate enhanced non-specific immune responses, with agranular and semi-granular hemocytes playing vital roles in shrimp immunity.

The use of probiotics, prebiotics, and synbiotics has also been proven to enhance shrimp growth, welfare, and disease resistance (Khanjani *et al.*, 2024). In intensive culture systems, probiotics contribute to controlling pathogenic bacteria through competitive exclusion, organic matter degradation, and pH stabilization (Liu *et al.*, 2023).

The observed average body weight (ABW) of 13.37 g at day 91 reflects optimal growth. Akbarurrasyid & Adibrata (2023) reported that ABW in intensive systems typically ranges from 1.61 to 21.1 g. Cahyono *et al.*, (2023) found an ABW of 21.33 g at day 72 in low-salinity ponds. The observed growth is likely influenced by the use of vitamin C, vitamin E, garlic extract, and probiotics. Vitamin C is essential for collagen synthesis and stress resistance, while vitamin E acts as an antioxidant, protecting cells from oxidative damage (Siswanto 2008). Garlic extract contains allicin, which acts as a natural antibacterial and immune booster. Hasrianda & Setiarto (2022) found that allicin activates non-specific immune responses by upregulating cytokine gene expression. Additionally, commercial probiotics containing *Lactobacillus* and *Bacillus* species help balance gut microbiota, enhance nutrient absorption, and improve water quality (Anwar *et al.*, 2016).

The feed conversion ratio (FCR) of 1.7 observed in this study reflects relatively good feed efficiency, although slightly above the company's ideal target. Arsad *et al.*, (2017) reported FCR values between 1.4–1.8 as typical for intensive *L. vannamei* farming. This result suggests that feed, environmental, and health management systems were effective, but improvements remain possible. Feed efficiency is also supported by probiotic supplementation, which enhances digestive enzyme activity, improves water quality, and suppresses pathogenic microbes.

A productivity level of 20.39 tons/ha achieved in this study is considered high for intensive systems. Daniel, *et al.*, (2024) reported an average productivity of 23.9 tons/ha in Banyuwangi under an intensive system with environmental and health management approaches. The high productivity observed here was supported by the combined application of immunostimulants (vitamins, minerals, and garlic extract) and probiotics, which contributed to the physiological resilience of the shrimp.

4.2 Water Quality

The water quality results showed that the application of quicklime (CaO) at 10 ppm, probiotics at 2 ppm, and dietary immunostimulants (vitamin-mineral mix and garlic extract at 2 ppm) played a crucial role in maintaining stable environmental conditions in the culture system. Although some water parameters deviated from optimal ranges—such as pH (7.21–9.12), TOM (up to 134 ppm), and TAN (up to 2.6 ppm)—shrimp performance remained optimal, as evidenced by the SR (91.01%), ABW (13.37 g), and FCR (1.7). These findings suggest that *L. vannamei* was able to adapt effectively under suboptimal conditions.

Quicklime functions as a buffering agent that increases pH and alkalinity, creating a mildly alkaline environment that enhances shrimp metabolism and reduces the toxicity of un-ionized ammonia (Boyd & Tucker 2012). Probiotic bacteria such as *Bacillus* spp. contribute to TAN and TOM reduction through organic matter degradation and nitrification, while also promoting microbial balance in the culture water (Martínez-Córdova *et al.*, 2023).

Biological water quality indicators further supported the success of the culture system. Total phytoplankton concentrations fluctuated considerably during the early and mid-culture stages, peaking at approximately 4.8×10^5 cells/ml. This pattern represents a typical dynamic in intensive systems, and the stability observed in the later stages reflects a balanced primary productivity. Probiotics such as *Bacillus subtilis* have been shown to stabilize phytoplankton communities by suppressing pathogenic microbes and maintaining ecological equilibrium (Zokaeifar *et al.*, 2012).

The average total bacterial count (TBC) reached 2.4×10^5 cfu/ml, while the total vibrio count (TVC) was 1.1×10^4 cfu/ml, resulting in a TVC/TBC ratio of 9%. This ratio indicates the dominance of non-pathogenic bacteria in the system. The low proportion of *Vibrio* reflects the effectiveness of probiotics

in suppressing pathogenic populations through nutrient competition, niche exclusion, and production of antibacterial compounds (Farzanfar, 2006). This microbial balance plays a vital role in maintaining shrimp health and survival.

5. Conclusions

Based on the results of this study, it can be concluded that although several water quality parameters exceeded ideal thresholds, the combined application of immunostimulants, probiotics, and 10 ppm CaO lime effectively maintained the health and performance of *L. vannamei*, as indicated by a survival rate of 91.01%, an average body weight of 13.37 g, a feed conversion ratio of 1.7, and a productivity of 20.39 tons/ha.

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