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Growth of *Litopenaeus vannamei* using Synbiotics Supplementation Diet in Outdoor Low-Salinity Ponds Concerning Water Quality Parameters and Phytoplankton Communities

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Abstract

This study aimed to evaluate the influence of probiotic supplementation using *L. bulgaricus* FNCC-004 and prebiotics sodium alginate on the growth of *Litopenaeus vannamei* shrimp, as well as water quality parameters and plankton density in a five tons circle outdoor pond for 30 days of rearing. Assessment was conducted on specific pathogen-resistant (SPR) shrimp. Shrimp weight, plankton density, and water quality, with samples collected from circular ponds. The probiotic *L. bulgaricus* FNCC–004 was prepared as feed supplementation by adding 2% sodium alginate into the incubated bacterial media. One kg of feed was mixed with 200 mL of media containing the alginate-probiotic mixture. Feeding frequency was four times a day. Water quality sampling involved temperature, salinity, pH, dissolved oxygen (DO), and light intensity, measured twice daily. Plankton density was determined using a haemocytometer under microscope. Specific growth rate (SGR) of shrimp was calculated based on initial and final weights. Results showed variations in water quality parameters throughout the day, indicating the dynamic nature of the aquatic environment. Plankton density analysis revealed variations in species and abundance over the study period. The specific growth rate of *Litopenaeus vannamei* shrimp was recorded at 17.44% per day. This study enhances understanding of the effects of synbiotics supplementation in the diet on shrimp growth and environmental conditions in aquaculture systems.

1. Introduction

The cultivation of *Litopenaeus vannamei* shrimp is one of the most profitable sectors in the global aquaculture industry. *L. vannamei* is highly valued for its wide market share both domestically and globally due to its high economic value and advantages such as disease resistance and high productivity rates. However, high-productivity Vannamei shrimp farming generates significant farming waste, primarily nitrogen waste produced through the degradation and decomposition of organic compounds (Emerenciano *et al.*, 2021).

Water quality deterioration in shrimp ponds is caused by the accumulation of high inorganic compound content at the bottom, unstable dissolved oxygen levels, and high toxin-producing bacteria, which create a toxic environment for *L. vannamei*. Sediments at the pond bottom originate from shrimp feces, uneaten feed, and molting (ecdysis). Such conditions lead to higher concentrations of harmful bacteria like Vibrio, which thrive in high organic matter and poor water quality (Alfiansah *et al.*, 2018). High organic waste from feed residue and feces in shrimp farming leads to accumulation and sedimentation at the pond bottom, necessitating a decomposition process. Effective water quality management is essential to maintain the culture media in good condition, as poor water quality can reduce *L. vannamei* shrimp production performance. Fluctuations in water quality, particularly temperature, can decrease *L. vannamei*'s appetite, increasing ammonia content in the rearing media, which is toxic to the shrimp (Bull *et al.*, 2020; Rasyidah *et al.*, 2024).

Increasing *L. vannamei* production often faces problems with the emergence of diseases from bacteria, viruses, fungi, and even parasites, leading to economic losses (Walker and Winton, 2010; Yadav *et al.*, 2020). Moreover, water quality is a critical factor in the success of *L. vannamei* shrimp farming activities. Water quality deterioration will make *L. vannamei* susceptible to disease attacks, even mass mortality (Brito *et al.*, 2014). Therefore, alternatives are needed to increase *L. vannamei* shrimp production by improving their health in an environmentally friendly manner, such as through the administration of immunostimulants (Azhar and Yudiati, 2023).

Several studies have proven that alginate immunostimulants in *L. vannamei* produce positive results. A few research revealed that using alginate in *L. vannamei* can stimulate and enhance non-specific immune responses, preventing WSSV infection (Yudiati *et al.*, 2019) and *Vibrio parahaemolyticus* AHPND (Azhar and Yudiati, 2023). Setyawan *et al.* (2021) also reported that adding alginate extract at a dose of 2 g/kg feed can increase Total Haemocyte Count (THC), Phagocytosis Activity (AF), and Total Plasma Protein (TPP). This study aimed to evaluate the influence of probiotic-prebiotic (synbiotics) supplementation using *L. bulgaricus* FNCC-004 and sodium alginate on the growth of *L. vannamei* shrimp, as well as water quality parameters and plankton density in aquaculture systems.

2. Material and methods

2.1 Materials

The materials was shrimp with 0.01 ± 0.00 g in weight. Shrimps were reared in 5 tons circular tank at the density of 86 ind.m^2 for 30 days. The salinity of media was maintained at low (4-6 ppt).

2.2 Methods

2.2.1 Preparation of probiotic *L. bulgaricus* FNCC–004 and prebiotic sodium alginate as feed supplementation

FNCC–004 was provided by the Laboratory of Tropical Marine Biotechnology, Faculty of Fisheries and Marine Science, Diponegoro University. Previously, all glassware, media, and sterilization materials were autoclaved and sprayed with 70% alcohol in a Laminar Air Flow under UV exposure (Guridi *et al.*, 2019). One colony of FNCC–004 cultured in De Man, Rogosa, and Shape Agar/MRS Agar media was resuspended in 100 mL of Nutrient Broth/NB (Merck, USA) and 5% MRS broth, then incubated for 24 hours at 37°C (Yudiati *et al.*, 2023; Yudiati *et al.*, 2021). A prebiotic-probiotic (synbiotic) mixture was formulated by mixing FNCC–004 and sodium alginate. Alginate was prepared by adding 2% into the incubated bacterial media. Feed weighing 1 kg was mixed with 200 mL of media containing the Alginate-probiotic mixture.

2.2.2 Experimental design

The shrimp used were specific pathogen resistant (SPR) from the CV Hadid Mukti Karya hatchery, stocked at a density of 86 shrimp/m² in circular ponds with a diameter of 3 meters and a water depth of 1 meter (1.2 meters total depth). Before stocking, acclimatization was done by placing the shrimp bags in the ponds to match the pond water temperature. Feeding frequency was four times a day at 05:00, 10:00, 15:00, and 22:00.

2.2.3 Water quality sampling procedure

Water quality sampling involved measuring temperature, salinity, pH, dissolved oxygen (DO), and light intensity. Data was collected two times a day at 8:00 AM and 4:00 PM.

2.2.4 Plankton density

Plankton algae are counted using a haemocytometer under a microscope at varying magnifications depending on their size. Larger forms are counted under low magnification, while smaller or more challenging forms are observed under higher magnification, using an immersion oil for accuracy. When larger plankton organisms are sparse, it is advisable to count them across the entire chamber bottom for reliability. However, in cases of high plankton density, counting only a portion of the chamber bottom, such as several diagonal fields, suffices to account for any irregularities while still providing representative data. This adaptable approach ensures accurate and reliable estimation of plankton density, tailored to the sample's characteristics (LeGresley and McDermott, 2010; Willén, 1976). Identification guides used for various common phytoplankton species included works by Genkal (2012); Kilham and Hecky (1988); Reynolds (1984).

2.2.5 Specific Growth Rate

The shrimp were randomly chosen from the population. Their weight was recorded on days 0 and 30 of the maintenance period using a scale. After completing the measurements, the weight data were used to determine the specific growth rate (SGR) as follows:

$$SGR\% = \frac{(LnWt - LnWo)}{t} x100\%$$

SGR = Specific Growth Rate
Wt = Final weight
Wo = Initial weight

3. Results

3.1 Water quality parameters

The water quality parameters measured at 8:00 AM and 4:00 PM showed variations (Table 1). At 8:00 AM, dissolved oxygen levels ranged from 5.58 to 6.9 mg L-^{1}, temperature from 26.8 to 29.3°C, light intensity from 22,300 to 61,000 Lux, pH from 7.8 to 8.02, and salinity from 4 to 6 ppt. At 4:00 PM, dissolved oxygen levels ranged from 6.13 to 7.16 mg L-^{1}, temperature from 29.2 to 32.3°C, light intensity from 4,370 to 20,400 Lux, pH from 7.9 to 8.66, and salinity from 4 to 6 ppt. These findings illustrate the fluctuations in water quality parameters throughout the day, providing insights into the dynamic nature of the aquatic environment.

3.2 Plankton identification and density

Base on Table 2, the plankton density measurements over 10, 20, and 30 days showed that Scenedesmus appeared only on day 30 (11.11 x 10^3 cell mL^{-1}), Anabaena only on day 20 (11.11 x x 10^3 cell mL^{-1}), Microcystis peaked on day 20 (33.33 x 10^3 cell mL^{-1}) but was absent on day 30, Cyclotella had the highest density on day 10 (425.66 x 10^3 cell mL^{-1}) and then decreased, Rhizosolenia remained constant (11.11 x 10^3 cell mL^{-1}), and Peridinium appeared only on day 20 (11.11 x 10^3 cell mL^{-1}).

3.3 Specific Growth Rate (SGR)

According to Table 3, the initial and final weights were 0.01 ± 0.00 and 1.87 ± 0.34, respectively. The specific growth rate was recorded at 17.44% per day.

Table 1. Water Quality Parameters

Sampling hours	Parameters				
	Dissolved Oxygen (mg L-^{1})	Temperature (ºC)	Light Intensity (Lux)	pH	Salinity (ppt)
8:00 AM	5.58 – 6.9	26.8 – 29.3	22300 - 61000	7.8 – 8.02	4 - 6
4:00 PM	6.13 – 7.16	29.2 - 32.3	4370 - 20400	7.9 – 8.66	4 - 6

Tabel 2. Plankton Identification and Density every 10 days of rearing

Plankton	Genus	Plankton Density (x 10^3 cell mL^{-1})		
		10 days	20 days	30 days
Green Algae	Scenedesmus	ND	ND	11.11
Blue Green Algae	Anabaena	ND	11.11	ND
	Microcystis	16.5	33.33	ND
Chrysophyta	Cyclotella	425.66	81.48	140.74
	Rhizosolenia	11.11	11.11	11.11
Dinoflagelata	Peridinium	ND	11.11	ND

ND : Not detected

Table 3. Weight of *Litopaneus vannamei*

Initial weight (gr)	Final weight (gr)	Specific Growth Rate (%)
0.01 ± 0.00	1.87 ± 0.34	17.44 %

4. Discussion

The variations in water quality parameters and plankton density observed in this study are critical for understanding the dynamic nature of aquatic ecosystems. Water quality is a critical factor in maintaining the health of aquatic environments (Rasyidah *et al.*, 2024). It influences the survival, growth, and reproduction of aquatic organisms. Table 1 presents the water quality parameters measured at 8:00 AM and 4:00 PM, showing significant variations throughout the day. These fluctuations align with the findings of Boyd and McNevin (2015a, 2015b); Boyd and Tucker (1998), who noted that diurnal variations in dissolved oxygen and temperature are common in aquaculture ponds due to photosynthesis and respiration cycles. The increase in dissolved oxygen in the afternoon could be attributed to photosynthetic activity, which peaks during daylight hours (Nzayisenga *et al.*, 2020). However, the higher afternoon temperatures may increase metabolic rates, leading to greater oxygen consumption (Ebeling *et al.*, 2006). The stability of salinity is crucial for shrimp growth, as *L. vannamei* is known to tolerate a wide range of salinity levels but thrives best in consistent conditions (Boyd and Pillai, 1985). The slight fluctuations in pH observed in this study are within acceptable limits for shrimp culture, as extreme pH levels can be detrimental to shrimp health (Yu *et al.*, 2020).

Plankton density is a key indicator of the primary productivity and overall health of an aquatic ecosystem. These findings are consistent with studies by Glibert (2017) and Visser *et al.* (2016), who observed that plankton populations are influenced by nutrient availability, light conditions, and predation. The peak in Microcystis density on day 20, followed by its absence on day 30, could be due to nutrient depletion or increased grazing pressure (Paerl and Otten, 2013). The constant presence of Rhizosolenia suggests a stable niche, likely supported by consistent environmental conditions. The high initial density of Cyclotella and its subsequent decrease may reflect an initial burst of nutrient availability, followed by competitive exclusion or resource limitation (Smayda, 1997). This pattern underscores the importance of monitoring and managing nutrient inputs to maintain balanced plankton communities, which are crucial for supporting higher trophic levels, including shrimp (Kudela and Gobler, 2012). The presence of Microcystis, a known harmful algae, on day 20 highlights the potential risks associated with nutrient imbalances. Harmful algal blooms (HABs) can have devastating effects on aquaculture operations, leading to mass mortalities and significant economic losses (Glibert, 2017).

This high SGR indicates optimal growth conditions, likely facilitated by the controlled water quality parameters and sufficient nutrient availability. The growth rate observed in this study is comparable to those reported by Gao *et al.* (2016) and Khanjani *et al.* (2020), who found that *L. vannamei* exhibited high growth rates under optimal conditions. The stability of key water quality parameters, such as salinity and pH, likely contributed to this favorable outcome (Ariadi *et al.*, 2019). Probiotics, as living microorganisms administered orally, lead to health benefits by altering the microflora in specific host compartments, stimulating innate, cellular, and humoral immune responses. Prebiotics, indigestible fibers, enhance gut bacteria, producing by-products that improve host health. Both modulate immunity, boosting aquatic animals' health (Akhter *et al.*, 2015). This remarkable growth rate, represented by a specific growth rate of 17.44% per day, underscores the positive impact of probiotic-prebiotic supplementation using *L. bulgaricus* FNCC-004 and sodium alginate on the growth of *L. vannamei* shrimp. These results are consistent with previous studies that have demonstrated the efficacy of probiotics in promoting growth and improving overall health in aquaculture species (Chiu *et al.*, 2021; Lin and Chen, 2022)

The findings of this study have several implications for aquaculture practices. Maintaining stable water quality parameters is essential for promoting the health and growth of cultured species. Regular monitoring of dissolved oxygen, temperature, pH, and salinity can help identify and mitigate potential stressors in the aquatic environment (Boyd and Pillai, 1985; Boyd and McNevin, 2015b; Boyd and Tucker, 1998). The dynamic nature of plankton populations highlights the need for careful nutrient management. Excessive nutrient inputs can lead to harmful algal blooms, while insufficient nutrients can limit primary productivity (Kudela and Gobler, 2012). Balancing nutrient levels is crucial for maintaining healthy and productive aquaculture systems. The high specific growth rate of shrimp under the conditions studied suggests that controlled environments with consistent water quality and adequate feeding regimes can enhance shrimp production. This information is valuable for optimizing aquaculture practices, particularly in regions where environmental conditions can vary widely.

5. Conclusions

Plankton density measurements further emphasize the significance of nutrient management and environmental stability. The high specific growth rate of shrimp under these conditions provides a practical reference for aquaculture practices, reinforcing the value of maintaining controlled and favorable environmental conditions to promote growth and productivity.

Ethics approval

No permits were required.

Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Author contributions

MLMF : sample collection, water quality sampling collection. AR and JS : research ideas, supervising, editing and writing.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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