

Analysis of Probiotic Application, Water Quality, Carrying Capacity and Production of *Penaeus vannamei* Vaname Shrimp Culture in Ponds

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Abstract

The research aims is to analyze the probiotic, water quality, and carrying capacity of vaname shrimp farming in sustainable ponds. The research location was Rhee Royal Vannamei Sumbawa West Nusa Tenggara, Indonesia. The research was carried out from September 2023 to February 2024. Vannamei shrimp were reared for 96 days in a pond area of 3,433±157 m² at a stocking density of 133±5 shrimp/m². Cultured probiotics were applied to the pond once every 3 days at a dose of 0.5 ppm in day of culture for 60 days and at a 1-1.5 ppm DOC from 60 to 96 days. The research method was randomized with two treatments and six replications. Carrying capacity was calculated using production carrying capacity. Data collection methods were observation, field, and laboratory measurements. The results showed that the T-test showed that applying probiotics and non-probiotics had no significant effect. Still, the productivity of probiotic plots was more stable and essential than non-probiotic plots. Probiotic plots produced 29.58% more than non-probiotic plots. Probiotics can improve water quality. The carrying capacity is 21,354 kg/ha. Environmental management is essential for the success of sustainable shrimp cultivation in ponds.

Introduction

Shrimp is one of the leading commodities with high economic and foreign exchange value. Shrimp is an important economic commodity that often faces production challenges due to pathogen infection, sub-optimal water quality and production variability. The intensification of Vaname shrimp farming (*Penaeus vannamei*) Boone, 1931 to increase production has led to several cases, including the emergence of various types of diseases and decreased water quality (Rifalda et al., 2023; Agrianti et al., 2022; Wahyudi et al., 2022).

According to Mustafa et al. (2019); Piamsomboon & Han (2022), one of the diseases that causes high mortality is Vibriosis, this disease is caused by an environment compatible with Vibriosis and poor water quality for shrimp farming. Among various pathogenic bacteria, *Vibrio harveyi* is the cause of severe disease in shrimp (Abdel-Latif et al., 2022).

The efforts that can be made to improve the water quality of the aquaculture environment and the growth of Vaname shrimp are through the administration of probiotics (Rakhfid et al., 2018; Norman et al., 2024). This statement is supported by the explanation that one

of the efforts of probiotics are beneficial live microbes that can improve growth performance, increase disease resistance, modulate host immunity and improve water quality, degrade organic matter, strengthen the host against pathogens, and could reduce pollutants, improve environmental quality to create healthy and sustainable pond conditions. (Liu et al., 2022; Wang et al., 2020).

Several studies on probiotics in shrimp farming have produced highly variable recommendations because the studies were conducted in laboratories with different production scales and in the field with different results. This condition is due to the effectiveness of bacteria influenced by several factors, such as culture conditions, administration method, dose, probiotic strain, and shrimp species; thus, inconsistent results regarding the effectiveness of probiotic treatments on shrimp survival and growth performance (Toledo et al., 2019).

Most of the literature reviewed suggests that probiotics can have positive effects on shrimp growth performance through several pathways, including enhancing host digestive capacity, improving gut histoarchitecture, increasing the prevalence of beneficial bacterial colonies in the gut, and providing several essential nutrients. Explanation by Jahan et al. (2021), Islam et al. (2024), and Muthu et al. (2024). Probiotics can enhance the growth performance of animals or shrimp by improving host nutrition, providing some essential nutrients (e.g., vitamins, fatty acids, etc.), and improving digestive function through extracellular enzymes (e.g., proteases and lipases). Probiotic bacteria also affect water bioremediation through direct uptake or degradation of organic matter or toxic metabolites in the culture medium or effluent.

(Wang et al., 2020). Several probiotic bacteria from the genera *Bacillus*, *Vibrio*, *Lactobacillus*, *Enterococcus*, *Aeromonas*, and *Arthrobacter* have been described to improve the growth and health of farmed shrimp (Chiu et al., 2021; Ai et al., 2022; Kim et al., 2022).

Growth and stability of shrimp production are strongly influenced by the shrimp farming environment in ponds. One way to improve the environment is by using probiotics. Shrimp aquaculture production must also be managed in a sustainable manner, based on the ability to produce, which can be calculated as the carrying capacity of the land. The concept of carrying capacity is very important in commercial fisheries resource management (Hilborn et al., 1995). The purpose of this study was to analyse probiotic application, water quality, carrying capacity, and shrimp production. The objective of culture is efficient, effective and sustainable production. For this reason, the productivity of Vannamei shrimp in ponds is used as an indicator of the success of improving culture carrying capacity, water quality and probiotic application. Good shrimp production will increase the world's food supply and welfare.

Shrimp Cultivation

Study Area and Time

The research site was located in the industrial farm Rhee Royal Vannamei in Sumbawa Regency, West Nusa Tenggara, Indonesia. Geographical location at coordinates 8°24'18"LS 117°14'09"BT. Map of the research site in Figure 1. The research period was September 2023 to February 2024.

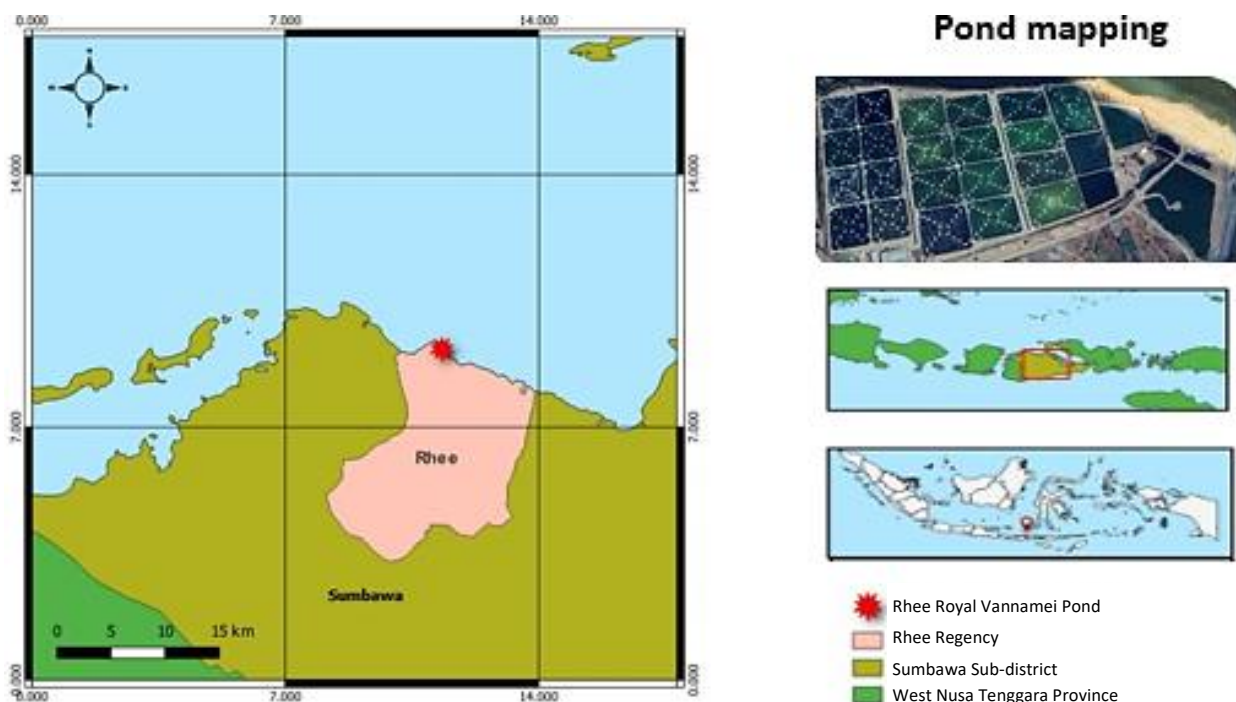


Figure 1. Map of the research location.

Materials and Procedures

Cultivation using Vannamei shrimp (*Penaeus vannamei*) post larvae 11 days, average initial weight 0.004 g and stocking density 133±5 shrimp/m² with a rearing period of 96 days. Pond preparation included plastic and plot cleaning, drying, pond construction improvement, water inflow, and wheel installation. Drying was carried out for 14 days to physically sterilize the pond bottom using sunlight. The establishment of plankton culture media water is done by adding micromineral and lime. Feeding was 4-5 times/day on the day of culture (DOC) 1-51 days and 5 times/day on DOC 52-98.

Probiotic Microbial Activation

The bacteria used are capable of breaking down organic matter such as *Bacillus subtilis* contained in aquazyme brand probiotic packaging. The probiotic culture medium consists of 65 grams of Aquazyme, 15 grams of vitamin B12, 15 grams of vitamin C, 200 grams of azomite, 1 liter of molasses, 200 grams of food, 35 liters of water. The preparation consists of mixing all the above ingredients in a container and aerating for 24 hours to increase the density of beneficial bacteria to a maximum of 10⁹ (Jefri et al, 2020). The majority of probiotic species that grew were *Bacillus* spp. microscopic checks were carried out before the probiotics were stocked to determine the dominance of harmful organisms such as protozoa in the probiotics. The probiotics were applied directly to the pond water media at a dose of 0.5 ppm per week at the start of rearing until the day of culture (DOC) 2 months and at a dose of 1-1.5 ppm at the end of rearing or DOC 60-96 days. Probiotics are added in the afternoon when the sun's rays are not too strong. Water microbiological measurements pond water samples of up to 100 µL were taken from each plot and then spread on TCBS media using a triangular stick. The media were then incubated for approximately 24 hours and the number of colonies observed and counted.

The water quality parameters measured were brightness with a 30 cm diameter black and white sechi dish (made alone). Salinity was measured with a refractometer trade merck Atago, temperature was measured with an alcohol thermometer with a scale of 0-100°C. pH was measured with a Eco pH Aquaria and DO with a DO meter Pro 20 YSI brand, ammonia, nitrite, and phosphate were measured with ammonia test kit Hanna hi3826, Merck nitrite test Kit, Phosphate test kit Merck.

Research Method

The research method used was a randomized design with two treatments and six replications. The number of plots with probiotic application was 6 plots and 6 plots without probiotics. The bacterial population

was calculated by total plate count (TPC). The variables observed in this study were average body weight (ABW), average daily growth (ADG), feed conversion ratio (FCR), survival rate (SR), productivity, and *Vibrio* sp. bacterial population.

Isolation of *Vibrio* sp and Probiotic

Vibrio sp. bacteria were isolated from 12 samples of pond water from 12 different pond locations. Sampling of pond water was done by holding the bottom of a sterile sample bottle. The sample bottle was dipped into pond water ±20 cm with the position of the bottleneck tilted down. Furthermore, it is stored in an ice box for analysis in the microbiology laboratory. Pond water samples of as much as 1 ml were put into a sterile test tube containing 9 ml of trisalt solution. The solution was stirred until homogeneous and marked 10-1. Take 1 ml of sample from the first tube sample and put it into 9 ml of trisalt. This process was carried out until a dilution of 10-4 was obtained. Taken 1 ml from each dilution, put into a petri dish, and made duplo. Next, TCBSA (Thiosulfate Citrate BileSalt Sucrose Agar) media was added. After the media hardened, it was incubated in an incubator at 34°C for 48 hours in an inverted position (Chau et al., 2011). The method used to calculate the number of *Vibrio* sp colonies is the total plate count. Colony growth was recorded from each Petri dish. The total plate count (TPC) of bacterial colonies at each dilution level was calculated using a colony counter (Radji, 2011). Calculation of Total *Vibrio* Count (TVC) (Ambat et al, 2022).

$$N = \sum C [(1 \times n_1) + (0,1 \times n_2)] \times (d)$$

Where, N= Number of product colonies, expressed in colonies/ml or colonies/gram; C= The number of colonies on all plates was counted; n1= The number of plates in the first dilution is counted; n2= The number of plates in the second dilution is counted; d= The first dilution is calculated.

Data Processing Method

Carrying capacity calculations consider growth or production rates and simultaneously consider species interactions with the environment in production carrying capacity, which is the stocking density at which harvests are maximized and also referred to as production capacity, as in this paper (Newell, et al, 2007; Ross et al, 2013; Filgueira et al, 2015). Similarly (Song et al., 2019), in intensive ponds, the carrying capacity of aquaculture can be calculated from the maximum production capacity, and the environment can accommodate the amount of waste load generated by aquaculture activities.

Cultivation performance data included productivity, survival rate (SR), food conversion ratio (FCR) and average body weight (ABW). Normality test as

a prerequisite for linear regression and paired samples t-test using Shapiro-Wilk. Hypothesis testing can be done if the data are normally distributed and homogeneous. One-way Analysis of Variance with 95% confidence level or one-way analysis of variance (ANOVA). The margin of error was 5%. Data were analyzed using Analysis of Variance (ANOVA) to determine the effect of each treatment. Hypothesis Formulation:

$H_0 = P > 0.05$ means no significant effect on production yield of probiotic and non-probiotic plots

$H_1 = P < 0.05$ means Significant effect on production yield of probiotic and non-probiotic plots

When the results of the ANOVA tests were significantly different, the Duncan test (DMRT) was used to determine the difference between treatments. to determine the best treatment. Descriptive analysis was also carried out by comparing the results with the theoretical basis or literature related to the issue under discussion. The research mechanism and results can be explained according to Figure 2.

Results

Bacteria

After ±12 hours of probiotic administration, foam will appear in the plot. The appearance of this foam indicates that floc bacteria have formed. The presence of foam also affects the color of the water, which becomes thicker and is used as a shade by the shrimp to protect them from high temperatures. The results of calculating the total bacterial probiotic density in the probiotic plots at 60 days DOC were $1.38-5.89 \times 10^9$ CFU/mL; at 93 days were $4.40-8.52 \times 10^9$ CFU/ml.

Vibrio density in probiotic plots at stocking age up to 60 days ranged from 2.6×10^2 to 1.8×10^3 CFU / ml and at age 93 from $2.6-1.31 \times 10^3$ CFU / ml. The non-probiotic plots ranged from 1.6×10^2 CFU/ml to 4.98×10^3 CFU/ml at 60 days of age and 4.51×10^4 CFU/ml at 93 days of age. Vibrio bacteria are the main cause of vibriosis disease, also because their abundance exceeds the threshold and continues to increase over time. The vibrio that commonly infects shrimp from hatchery to grow out in ponds is *Vibrio harveyi* (Haliman et al, 2023).

Production

Evaluation of production as the end result of maintenance requires analysis of Average Body Weight (ABW), Survival Rate (SR), and Feed Conversion Ratio (FCR). Feed consumption accounts for more than 60% of total costs. The recapitulation of the cultivation results is in the following Table 1.

The average ABW of the probiotic plots increased by 29.58% and the FCR by 13.88%. Except for SR in non-probiotic plots increased by 1.01%. Productivity in probiotic plots was 12.85 ± 1.85 kg/ha while in non-probiotic plots it was 9.8 ± 4.8 kg/ha. Average production of probiotic ponds tonnes and non-probiotic ponds tonnes or an increase of 29.58% The graph of the productivity value of shrimp farming can be seen in the following Figure 3.

Figure 3 above shows the highest productivity in plots with probiotics at 15.8 t/ha. The lowest productivity in the non-probiotic plots was 4.5 t/ha. The difference in productivity between the two types of plots is due to disease infections that affect shrimp during the rearing period.

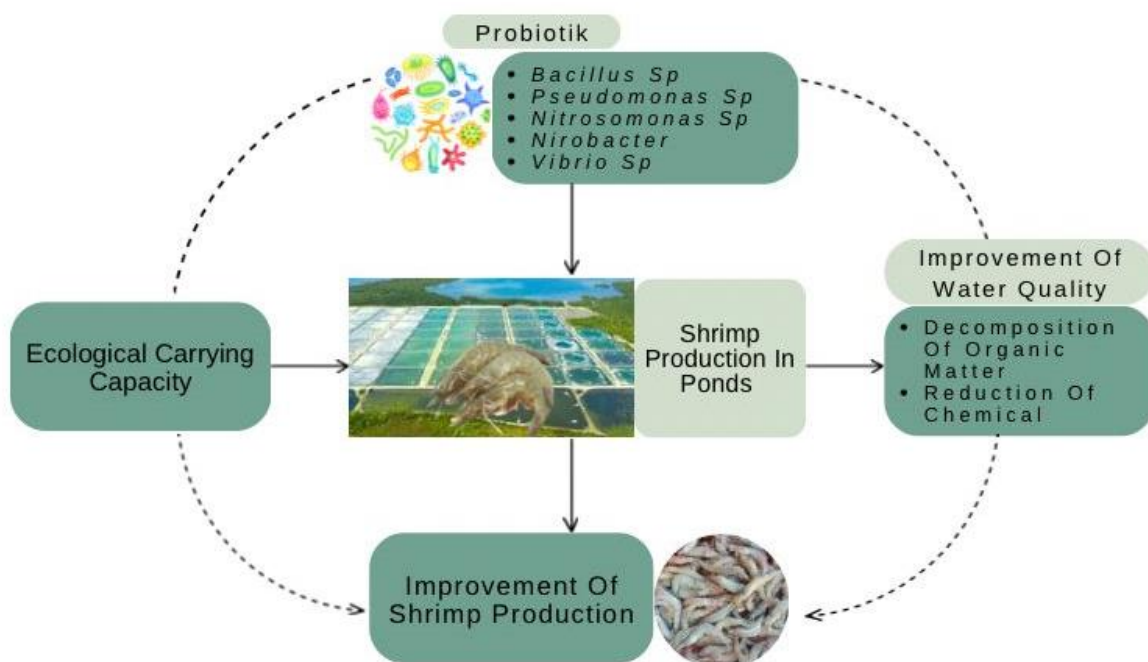


Figure 2. Research scheme for achieving results.

Aquaculture Water Quality

The results of the water quality measurements are shown in Table 2 below.

The results of the water quality tests of the probiotic plots showed low variations of several main parameters in the shrimp pond water. Ammonia 0.02 ± 0.1 mg/L and Nitrite 0.055 ± 0.025 mg/L. Non-probiotic plots Ammonia 0.03 ± 0.1 mg/L and Nitrite 0.1 ± 0.8 mg/L. Nitrite threshold of 0.25 mg/L, so that low nitrite levels are suitable for shrimp culture, H₂S presence is expected to be no more than 0.002 mg/L. Other parameters such as DO, temperature, salinity, and brightness of probiotic and non-probiotic plots were relatively similar.

Carrying Capacity

Carrying capacity is the optimum sustainable production capacity, one method is the production capacity method. Production data for 5 cycles coinciding with the 15-19th cycles are shown in Table 3 below.

The average amount of average production = 21,354 kg/ha. The detailed production data per cycle is shown in Figure 4 below.

Discussion

The success of shrimp production in ponds is largely determined by the quality of the culture medium water, and probiotics can be used to improve the quality. For sustainable production, the carrying capacity of the land must be considered.

Vibrio

Pathogenic bacteria can attack shrimp when they are stressed and weak. One of the pathogenic bacteria commonly found in shrimp farming is *Vibrio* sp. (Anton et al., 2020). *Vibrio harveyi* bacteria can infect shrimp at various stages, from nauplii in hatcheries to adults in grow-out ponds (Saulnier et al., 2000). As a result of infection with these pathogenic microorganisms, farmed shrimp experience mass mortality, resulting in high economic losses (Saputra, 2023). Some research results show that safe levels of *Vibrio* bacteria are 3.5×10^4 to 5×10^4 CFU/mL (Widiyanto et al, 2019); 6.2×10^2 to 2.6×10^3 CFU/mL (Idami and Nasution, 2020); 10^4 CFU/ml (Asmiyanti et al., 2024); 10^3 CFU/mL (No.75/PERMEN-KP/2016). (Ambat et al., 2022) and 10^4 CFU/mL (Kurniawan et al., 2014). Non-probiotic plots

Table 1. Production data for both types of parcels

Plots	Probiotic plots				Plots	Non Probiotic			
	ABW (g/shrimp)	SR%	FCR	Productivity (kg/Ha)		ABW ((g/shrimp)	SR ((%)	FCR	Productivity (kg/Ha)
A1	18.87	45	1.1	11,293	B1	9.71	57	1.3	7,686
A2	17.24	52	1.1	12,017	B2	13.62	81	1.1	14,054
A3	19.23	59	1.2	14,665	B3	10.94	30	1.5	4,515
A4	19.61	53	1.1	12,733	B4	19.46	39	1.3	9,712
A5	16.67	72	1	15,797	B5	19.16	54	1.2	13,270
A6	15.50	51	1	11,020	B6	10.64	79	1	10,607
Average	12,92	55,33	1,08	12,92	Average	9,97	56,66	1, 23	9,97

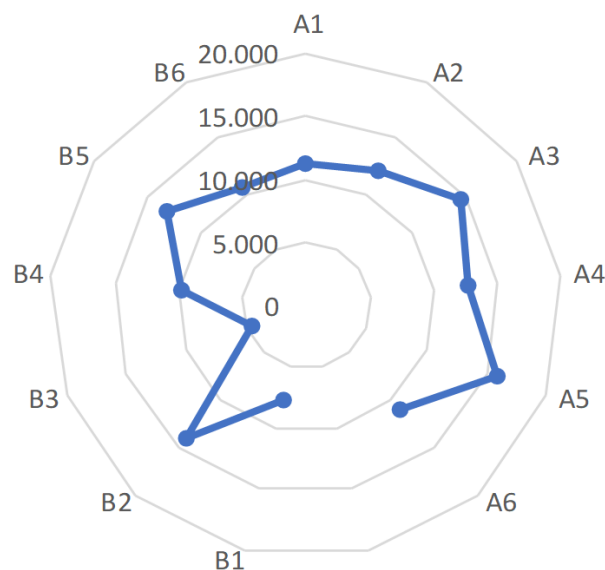


Figure 3. Productivity values, A= probiotic application, B= non probiotic.

had higher *Vibrio* density and range than probiotic plots. This suggests that some plots were affected by disease. *Vibrio* concentrations need to be assessed regularly as their growth can increase rapidly, reaching a population of 10^9 within 24 hours (Kurniaji et al., 2020). Improving water quality to reduce the risk of *Vibrio* can also be done by regularly changing the water and removing sediment from the bottom of the pond (Kamil et al., 2023).

Probiotic

According to Sumardi et al. (2019); Lilis and Adawiyah, (2021), probiotics have benefits that can be used to control host and environmental pathogens, stimulate shrimp immunity, and improve water quality. (It is also known that probiotics can also play a role in preventing these pathogenic bacteria in the digestive tract. The successful use of probiotics is characterized by the growth of flocs, which help to maintain water quality and can also reduce feed conversion ratios. (Lu et al., 2022; Gompi et al., 2023).

The concentration of probiotic bacteria 10^9 CFU/ml is safe for tiger shrimp culture (Muliani, et al., 2005). vaname shrimp culture probiotic bacteria concentration is safe 10^8 CFU/mL (Irianto, 2003). Concentrations of 10^7 CFU/ml have not been able to suppress the development of *Vibrio harveyi* (Ihwan, 2019), levels of 10^7 - 10^8 CFU/mL (Jefri et al., 2020) 10^6 CFU/mL (Pakaya et al., 2022). Pond waters range from 10^3 - 10^4 CFU/mL and marine waters 10^3 - 10^6 CFU/mL (Husaeni and

Sudarmayasa, 2018). The type of bacteria growing affects the vibrio density (Mustafa, 2019; Khanjani, 2024). Probiotics, particularly *Lactobacillus sp.*, were able to suppress the population of *Vibrio parahaemolyticus*, increasing the survival rate of Vaname shrimp to 86.67% (Amiin et al., 2023). (The ability of probiotics to suppress the number of *Vibrio sp.* bacteria is also influenced by the combination of bacteria contained in the probiotics. In multi-strain probiotics, such as *Bacillus sp.*, *Pseudomonas sp.*, *Nitrosomonas sp.*, *Aerobacter sp.* and *Nitrobacter sp.*, the effect on shrimp can be enhanced better than with mono-strain probiotics. (Toledo et al., 2019). According to Chrisnawati (2018), probiotics can increase the survival value of Vaname shrimp by 77% compared to without probiotic treatment. This condition causes some researchers to have different results, as many factors are influenced and the research media is carried out.

Based on the statistical test of normality obtained by KS table >D count, it is concluded that the data are normal. The homogeneity test obtained the value of F count >F table, the results obtained both treatments have unequal variance or not homogeneous. Based on this, the t-test was used to determine the effect of the two treatments: Two-Sample Assuming Unequal Variances (t-test). This decision is based on the provisions that if t count < t table, or p value > α , then H_0 is accepted. Based on the results of the T-test which shows that t count of 1.788 is less than the t table value of 1.860 and p value of 0.056 is greater than α which is 0.05, it can be concluded that there is no significant

Table 2. Results of water quality measurements

Parameter	Unit	Measurement value			Reference
		Probiotic plot	Non-probiotic plot	Standard	
Physics					
Brightness	cm	42±12	46±16	37±7.5	Ariadi et al., 2023
Salinity	g/L	33±3	33±3	21± 6	Anita et al., (2017)
Temperature	°C	28±1,7	28±1,7	29±1	Ariadi et al. (2023)
pH	-	7,9±0,3	7,9±0, 6	8±0.5	Jaffer et al., 2020
Dissolved Oxygen (DO)	mg/L	5,4±1,2	4,7±1,0	>3,54	Araujo et al., 2024
Alkalinity	mg/L	112-153	126-167	130 ± 30	Rifalda et al. (2023)
TOM	mg/L	108±15	108±15	128±31.5	Pratiwi et al., 2024
Amonia	mg/L	0.02±0.1	0.03±0.1	0.01	Araujo et al., 2024
Nitrit	mg/L	0,055±0,025	0.1±0.8	<0.2	Supriatna et al., (2020)
H ₂ S	ppb	<0,14	<0,14		SNI, 2006
Phospat	mg/L	0.007 – 0.01	0.3±0.1	0.1	SNI, 2006
Plankton	Ind/mL	15±6.10 ⁴ -	19±9.10 ⁴ -	100.10 ⁴	Anjaini et al., (2024)
Total vibrio count (TVC)	CFU/mL	0,35.10 ⁴	5.10 ⁴	6,2×10 ² - 2,6×10 ³ CFU/mL	Idami dan Nasution, 2020

Table 3. Total production per cycle

Siklus ke	Production (kg/ha)		
	Minimum	maximum	Average
15	26	40	34,81
16	21	28	23,82
17	10	25	17,75
18	12	25	18,25
19	7	16	12,14
Average			21,354

effect of productivity differences between probiotic and non-probiotic plots.

Probiotics maintain shrimp health by suppressing the growth of pathogenic bacteria that cause disease and increasing the growth of beneficial bacteria in the pond, helping to maintain water quality and shrimp health. The ability of probiotics to prevent/reduce the potential for disease through quorum quenching mechanisms. Pathogenic bacteria that attack shrimp to cause disease, such as *Vibrio sp.*, must undergo a quorum sensing mechanism. Quorum sensing is a way of communicating between bacterial populations to express their activities. In sufficient numbers, these bacterial populations will produce compounds that are toxic to shrimp. To improve water quality, probiotics can be used as bacteria that disrupt quorum sensing, that is, quorum quenching by disrupting the communication pathway between bacteria by damaging signalling molecules, inhibiting the production of signaling molecules or sabotaging the detection of signalling molecules (Zulfikar, 2019). Probiotics can improve shrimp's immune response and health status by reducing pathogenic microbes and maintaining low levels of ammonium/ammonia and nitrate in the water (Muthu et al., 2024). Lysozyme is an antibacterial agent and, as an opsonin, can facilitate the phagocytosis process by binding to the bacterial surface (Liu et al., 2016).

Farming Performance

FCR is calculated by dividing the total amount of feed consumed by the weight gain of the shrimp during the culture period. The lower the FCR value, the better the feed is utilized for shrimp growth. The average FCR of white shrimp diets is shown in Table 1. The feed conversion ratio (FCR) of shrimp was low in all treatments, but the average FCR was lower when

probiotics were used. Survival and FCR increased in shrimp fed probiotics. (Toledo, et al 2019)

However, the results of the field data (Table 1) show that the range of productivity of the probiotic plots is more stable and higher than that of the non-probiotic plots. Field conditions showed that the number of non-probiotic plots infected by disease was higher, resulting in lower productivity. In terms of SR, the non-probiotic plots are more variable, even the lowest SR is 30%, although there is the highest SR of 81% (Arsad et al., 2017). The SR value is classified as good when it reaches >70%, medium category 50-60% and low <50%. In terms of ABW and FCR, the results show better probiotic plots. This condition is in line with Merdekabasuki et al. (2023) that the provision of probiotics will be able to increase absolute weight growth and total shrimp production. According to Toledo et al. (2019) Meta-analysis was conducted to evaluate the effect of probiotics on shrimp growth performance, increase specific growth rate and improve feed conversion ratio.

Water Quality

Increased production increases sedimentation and feed accumulation at the bottom of the pond, thereby reducing dissolved oxygen levels and toxic gases (Dewi et al., 2023), dramatically increasing phytoplankton growth (Husna, 2023) and pathogenic bacteria, particularly *Vibrio sp.* (Mustafa, 2019). Water quality can also reduce shrimp appetite, slow growth, increase susceptibility to disease and even cause mass mortality (Suhendar et al., 2020). Ponds treated with probiotics had better ammonia levels and other parameters than ponds not treated with probiotics. Ammonia levels in probiotic ponds were 0.02±0.1 mg/L and non-probiotic ponds were 0.03±0.1 mg/L. The low concentration of ammonia in the culture media treated with probiotics was due to the nitrification and denitrification processes

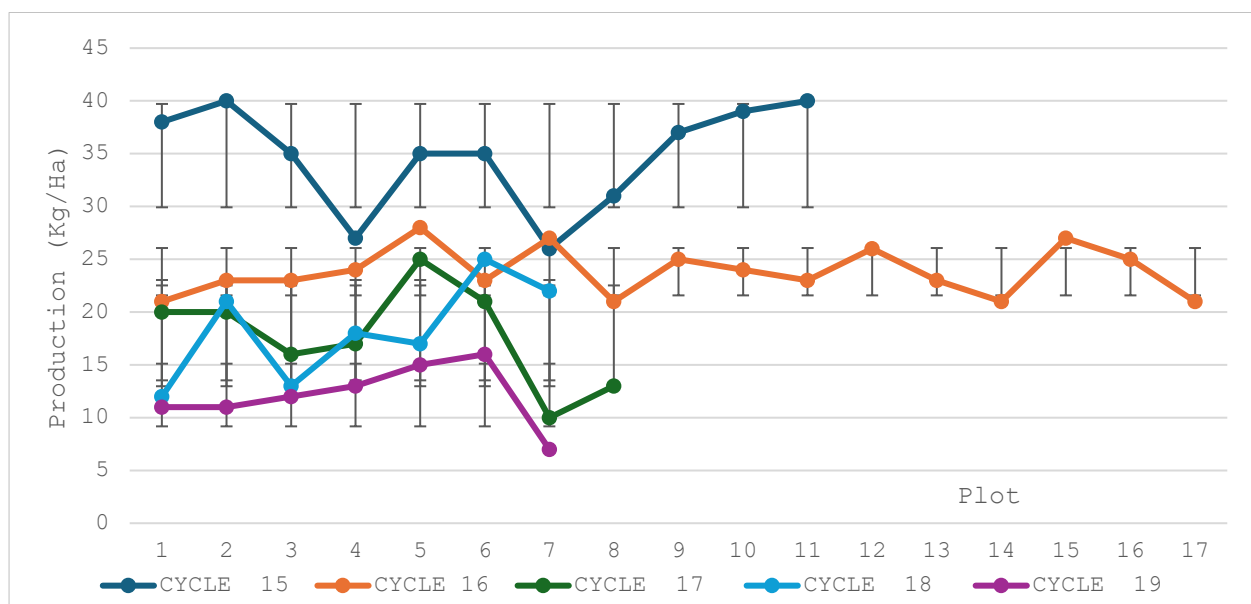


Figure 4. Production per cycle.

by the bacteria *Nitrosomonas sp.* and *Nitrobacter sp.*, which are owned by the probiotics. Therefore, the ammonia reduction process was faster than without the addition of probiotic bacteria (Chrisnawati, 2018). Meanwhile, Miranda's (2019) showed a lower amount of organic matter in the treatment of probiotic administration with a three-day interval. Based on Table 3, it is known that probiotic plots have better average water quality levels and the production obtained is also better.

Carrying Capacity

Carrying capacity analysis is used to optimise sustainable production and is usually the key metric that reflects the limits of growth rates and production capacity. Ecological carrying capacity is a broader concept than just production capacity, and takes into account the interaction of species with the environment, and together supports the use of other species for production purposes [Newell, et al., 2007; Ross et al., 2013]. Cultivation carrying capacity is the level of ecological suitability that can accommodate the maximum capacity of a biomass or waste load in the cultivation ecosystem (Wafi et al., 2021). Based on the analysis of the data and methods used, the average production in five cycles is the carrying capacity, namely 21,354 Kg/ha and a maximum of 40 tonnes/ha. These results show that the production capacity in this pond unit can still be increased by balancing and improving the facilities, infrastructure and technology used.

Conclusion

The relationship between the use of probiotics and non-probiotics was not significantly different. However, the performance of cultivation and the amount of production using probiotics, water quality and is better. Production can still be increased according to the carrying capacity of the aquaculture area.

Ethical Statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Central Fisheries Research Institute (SUMAE) (Protocol Number: 27-2956). All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

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Author Contribution

First Author: Conceptualization, Software, Data Curation, Formal Analysis, Validation, Writing -review and editing; Supervision, Resources;

Second Author: Conceptualization, Resources, Supervision, Writing-Review & Editing;

Third Author: Conceptualization, Supervision, Project Administration, Formal Analysis.

Fourth Author: Conceptualization, Data Curation, Formal Analysis, Investigation, Software, Methodology, Visualization, Resources. Validation, Draft Preparation, Project Administration and Writing-original;

Fifth Author: Writing-Review & Editing, Visualization, Validation;

Sixth Author: Funding Acquisition, Project Administration, Resources, Writing -review and editing;

Seventh Author: Supervision, Investigation, Project Administration, Resources.

Eighth Author: Draft, Data Curation, Writing-Review & Editing.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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