

Effectiveness of Different Probiotic Application on Vannamei Shrimp Productivity

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ABSTRACT Vannamei is a superior commodity of aquaculture in Indonesia. However, production of vannamei only reached 91.5% of the 450.000 tons revised target. Several factors are the cause of the target not being achieved. One of which is a disease outbreak. The use of probiotics as an application that improves the health status of shrimp is a better choice than the use of antibiotics for treatment. The effectiveness of the application of probiotics to control pathogenic microorganisms is highly influenced by the type of bacteria used. This research was conducted to examine the efficacy of using two different types of probiotics with different bacteria compositions on the productivity of vannamei shrimp. The 2.200 m² pond plots shielded with low-density polyethylene (LDPE) were used. The stocking density was 250 fry.m⁻². The vannamei shrimp were fed with an artificial diet containing protein from 30-40%, fat form 5%, fibre form 4%, and water content form 11% during rearing. In preparation and maintenance of water management, probiotics A and B. were applied. The results showed that the use of type B probiotics reached better productivity than type A.

Keywords: Financial analysis; probiotics; vannamei productivity

INTRODUCTION

Vannamei shrimp (*Litopenaeus vannamei*) is an essential economic commodity because of its fast growth, high survival rate, being not susceptible to disease, and being widely cultivated throughout the world (Santanumurti *et al.*, 2019). 40% of fishery products produced in Indonesia are sourced from white shrimp farming (Hadie & Hadie, 2017).

Intensive and super-intensive shrimp farming activities using high density and feeding increased feed residues and shrimp faeces, decreasing water quality, such as increased organic matter and pathogenic ammonia organisms. This decrease in environmental quality causes shrimp to become stressed and susceptible to disease. One disease that often attacks shrimp is Vibriosis, caused by bacteria from the genus *Vibrio*. White shrimp attacked by this disease can cause death up to 100% (Satyantini *et al.*, 2019).

The use of probiotics as an application that improves the health status of vannamei is a better choice than the use of antibiotics for treatment. In addition, antibiotics can have a resistant effect on cultured vannamei (Rume *et al.*, 2012). Probiotics protect vannamei by producing chemical compounds that are bactericidal and bacteriostatic against other bacterial populations.

Using probiotics to control pathogenic microorganisms is influenced by the bacteria used. The environment influences the life of bacteria. Bacterial populations in the environment require different nutrition and tolerance of physico-chemical parameters (Rume *et al.*, 2012). Probiotic bacteria obtained from the aquaculture environment are expected to have certain advantages compared to probiotic bacteria isolated from other sources.

Bacterial products originating from ponds and returned to ponds are expected to be more adaptable, reproduce, and carry out their roles (Muliani *et al.*, 2008).

Previously, PT Logigo Jaya Raya had not applied probiotics. The average yield per pond is low (SR 46.5±2.5%, ABW 14±0.7g, and biomass 3.486±98 kg). After one cycle of administration of Probiotic A, there was an increase in the value of SR, ABW, and Biomass. This study compares probiotics A and B (recommended by suppliers) and their effect on the productivity of vannamei cultivated at PT. Loligo Jaya Raya, Sidoarjo.

MATERIALS AND METHODS

This research was conducted from September to December 2020 at PT Loligo ponds, Sidoarjo, East Java (at ponds 1,2,3,4). The ponds measuring 2.200 m² (44x50x2 m³) used were protected with Low-Density Poly Ethylene (LDPE). The treatments tested were the application of probiotics A and B (with different bacterial compositions and application methods) on the productivity of vannamei. Cultivation was carried out up to DOC 90.

Pond preparation

The stages of pond preparation are pond drying, liming, lining repair, and pond construction. Pond preparation includes cleaning dirt and plankton that settles or moss-leveling the ground and resetting the slope towards the central drain. Administration of dolomite (CaMg (CO₃)₂) and quicklime (CaO) at a dose of 2 kg m⁻² and allowed to stand for three days.

Water treatment

Water is transferred by pump from the reservoir to the treatment tank until the water level is 50 cm. Subsequently, the treatment was carried out with a dose of 60 mg l⁻¹

chlorine and then adding water to a level of 110 cm. After two days, the chlorine content was tested. After the water is neutral, CaCO_3 is added at 50 mg-l. The next day, 1 mg l^{-1} of probiotic A and B was added continuously for three days in the morning (at 07.00 AM).

Stock density

The vannamei fry was stocked with SPF (Specific Pathogen Free) certified PL 11 stage. The fry was spread in the morning and previously acclimatized for 30 minutes. The stocking density of the fry is 250 m^{-2} ($550.000 \text{ pond}^{-1}$).

Feed management

The first 30 days of giving pellets are based on a blind feeding program, at DOC 31 and up to DOC 90 based on the anco program where the sampling net has caught the shrimp. The feed used is artificial with three types: fine crumble, crumble, and pellet. Fine crumble feed was used at the beginning of the rearing period until DOC 30. Then DOC 29 was replaced with crumble feed. For adaptation, the feed is mixed first, with the composition of feed fine crumble and crumble at DOC 29 (75% : 25%), DOC 30 (50% : 50%), and DOC 31 (25% : 75%) and DOC 32 (100% crumbles). However, after DOC 30, a sampling of shrimp growth was carried out. Based on the sampling results, the estimated shrimp biomass in the plots can be calculated so that the next feed requirement can be calculated through the FR determination value of the feed program. The FCR (Food Conversion Ratio) was calculated after the vannamei was harvested. This is to determine the effectiveness of the feed, and the effect of the probiotics applied.

Water management

Siphoning and water changes are intended to remove leftover feed and shrimp or plankton that settles at the pond's bottom. Wasted pond water is replaced with water that has been sterilized in the reservoir. Two different probiotic bacteria, namely probiotic A and probiotic B, improved water quality. In ponds 1 and 2, probiotic A was used, while in ponds 3 and 4 were probiotic B. Screening of probiotic A was carried out to identify the types of bacteria contained in it to compare its performance against the bacteria contained in probiotic B. Probiotic A is a liquid probiotic product whose application can be directly stocked in waters. Probiotic B is liquid, and the application must be fermented first, which aims to increase the number of bacteria. Procedure fermentation of probiotic B is for every 1 litre of probiotic B added 1 kg pellet DO and 1 kg of molasses.

Table 1. Bacteria composition, application method, and density of probiotics.

	Probiotic A	Probiotic B
Bacteria composition	<i>Pseudomonas spp</i>	<i>Pseudomonas putida</i>
	<i>Pseudomonas pseudoalcaligenes</i>	<i>Aspergillus niger</i>
	<i>Bacillus spp</i>	<i>Bacillus subtilis</i>
	<i>Acinetobacter calcoaceticus</i>	<i>B. lichenformis</i>
		<i>B. coagulans</i>
		<i>B. megaterium</i>
		<i>B. polymixa</i>
Application method	Non fermented	Fermented
Density	$3.0 \times 10^8 \text{ CFU ml}^{-1}$	10^6 CFU ml^{-1}

After the ingredients are homogenized, add seawater up to 100 litres and aerate for 24 hours.

Probiotics A and B were applied at 07.00 AM. Probiotics A and B were applied every five days. But at DOC 75, probiotics are given every two days at a dose of 2 mg-l until harvest time. A comparison of the composition of probiotics A and B can be seen in Table 1. Water quality was measured routinely in the morning and evening, including pH, salinity, temperature, DO, ammonia and nitrite.

Growth measurement

Growth was carried out by sampling at DOC 35 and after that every seven days until harvest to determine the growth and condition of shrimp. Sampling using nets, carried out every ten days, in the morning. The calculated data includes ABW (Average Body Weight), population, biomass.

Harvest and Post Harvest

Harvesting is done at DOC 91 with a net that is stretched (like a trawl) and pulled on both sides so that the shrimp collects on the side of the pond. Shrimp are lifted using a harvest basket. Then the vannamei are sorted to separate in size.

RESULTS AND DISCUSSION

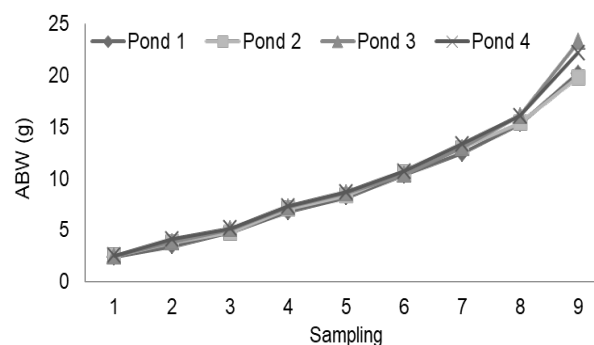


Figure 1. The average body weight of shrimp at 90 days of rearing.

Shrimp sampling to determine Average Body Weight (ABW) values was carried out at DOC 35, 42, 49, 56, 63, 70, 77, and 84. While at DOC 91, samples were counted at harvest. Until the seven samplings, the ABW values in ponds 1,2,3 and 4 were almost one line or showed no different values. In the 8th sampling, the growth of vannamei shrimp began to show a difference. At DOC 91, the ABW in ponds 3 and 4 is higher than ponds 1 and 2. Organic matter will increase with the age of shrimp rearing, which comes from the

remaining feed and the rest of the shrimp metabolism plankton. The ability of bacteria to be applied through probiotics is very influential. When the amount of organic matter that must be overhauled is beyond the limits of the ability of the bacteria, the water quality will deteriorate (Effendi, 2003).

Table 3. shows that the values of ammonia and nitrite in ponds 1 and 2 are higher than in ponds 3 and 4. Table 1 shows the composition of bacteria contained in the tested probiotics A and B. Both have almost the same number of *Bacillus*. *Bacillus* sp. is the most dominant bacteria in both probiotic products. This is based on several beneficial properties of *Bacillus* sp., namely the ability to live and grow in a wide range of environmental conditions, produce enzymes in large quantities, fight pathogens by inhibiting their growth, and competitively. *Bacillus* sp. also have thick cell walls to live longer, are resistant to dehydration, and are resistant to temperatures up to 60 °C (Decamp & David, 2005). *B. subtilis* produces the enzyme bacitracin. *B. lichenformis*, *B. coagulans*, *B. megaterium*. *B. polimyxa* produces the enzyme polymyxin. Soeka et al. (2011) stated that *Bacillus lichenformis* could produce several extracellular enzymes: amylase, aminopeptidase, metal proteases, lactam endo-N-acetylglucosaminidase, and lipase. These bacteria are also resistant to alkaline environments.

In cultivation, two factors must be considered to create a stable microorganism community: stochastic and deterministic factors (Rahayu, 2011). The deterministic factor is related to the dose and the response caused by the microorganism, such as its effect on water quality. At the same time, the stochastic factor is the opportunity for microorganisms to enter at the right time and environment to live. Probiotic A has a density of 3.0×10^6 CFU^{ml}, while probiotic B has a density of at least 10^6 CFU^{ml}. In their life cycle, bacteria reproduce asexually by dividing. The work of new bacteria is seen in the difference in the rank of log units. Although probiotic A and probiotic B have the same log unit rank, the application of probiotic B is fermented first, so, indeed, the rank of log unit density of bacterial colonies increases, and probiotic B has a higher density than probiotic A. with deterministic factors that influence. Probiotic A in the maintenance preparation stage is given for three days, and the maintenance application is given once a week according to the product usage recommendations. Probiotic B in the early stages of preparation was given for 13 days, and the application during maintenance was

Table 2. Pond productivity with differences values.

Parameters	Pond 1 (A)	Pond 2 (A)	Pond 3 (B)	Pond 4 (B)
Pond area (m ²)	2.200	2.200	2.200	2.200
Density (shrimp m ⁻²)	250	250	250	250
Survival Rate (%)	69.4	64.9	76.3	69.2
ABW (g)	20.23	19.86	21.6	22.9
Biomass (kg)	7.721.791	7.089.027	9.064.440	8.715.740
FCR	1.42	1.44	1.31	1.34
Total feed (kg)	10.964.943	10.208.199	11.874.416	11.679.092
population (shrimp m ⁻²)	173.5	162.25	190.75	173
Productivity (kg m ⁻²)	3.51	3.22	4.12	3.96

every day (stochastic). These two factors are the most likely to influence the influential work of probiotics outside of the bacterial species that affect them. A high value of feed efficiency indicated the shrimp response better to feed, so it was indicated by the growth of the shrimp (Anwar et al., 2016).

The increase in ABW of shrimp was also influenced by CaMg (CO₃)₂ and CaO, which were applied during the preparation and cultivation of shrimp. Dolomite and quicklime lime do not directly affect the growth of shrimp but can meet the needs of calcium and minerals when moulting and the hardening of the skin when moulting. The faster the recovery process of moulting shrimp will increase shrimp growth because after moulting, the shrimp's appetite will increase to satisfy its decreased appetite before moulting (Yunus et al., 2020).

Table 2 shows differences in survival rate values in ponds 1, 2, 3, and 4. The feed conversion ratio is one of the parameters that can measure the efficiency of using feed-in shrimp farming: the lower the FCR, the more efficient the use of feed. On the contrary, the higher the FCR, the more wasteful the feed's use in increasing the cultured shrimp's weight (Zainuddin et al., 2019).

The presence of microorganisms in the vannamei shrimp's digestive tract allows it to help digest food that enters the body (Zainuddin et al., 2019). Novitasari (2017) stated that mixing feed with *Bacillus* sp. bacteria can increase the growth of vannamei shrimp. *Bacillus* sp. into the feed with the right amount can suppress FCR.

The addition of probiotics can reduce the population of *Vibrio* sp. This study also shows the same results. This follows the opinion of Soundarapandian et al. (2010) and Yudiati et al. (2010), who reported that probiotic bacteria-derived from shrimp culture in water were potential and can suppress the growth of pathogenic bacteria, including *Vibrio* sp. Ammonia-degrading bacteria *Nitrosomonas eutrophica* and *Nitrobacter winogradsky* and organic matter-decomposing bacteria *Paracoccus pantotrophus* and *Bacillus* sp. were applied simultaneously to increase the survival rate by 6-7% but did not increase the growth of white shrimp (*L. vannamei*) tocolans. Giving probiotics can also reduce the total population of *Vibrio*, total bacteria.

The bacteria *Pseudomonas putida* used have not provided a consistent and significant effect on BOT, NO²-N, PO⁴-P, and NH⁴-N, but have a significant effect on H²S at the end

Table 3. Water quality measurement results during the vanamei shrimp rearing.

Parameters	Pond 1	Pond 2	Pond 3	Pond 4
Salinity (g l ⁻¹)	28-32	28-31	28-32	28-32
pH	7.6-8.2	7.6-8.1	7.6-8.2	7.8-8.2
DO (mg l ⁻¹)	3.4-8.3	3.4-8.7	3.9-7.7	3.7-7.9
Temperature (°C)	29.1-30.5	29.2-31.8	29.2-31.9	29.4-32.1
Ammonia (mg l)	0-0.5	0-0.5	0-0.25	0-0.25
Nitrite (mg l)	0-0.5	0-0.5	0-0.25	0-0.25

of the study on increasing shrimp survival rate. Probiotic bacteria can develop and suppress the growth of *Vibrio* sp both in the digestive tract and in shrimp rearing media. Furthermore, it was stated that the administration of probiotics *Bacillus* and *Pseudomonas* with a density of 10⁸ cells/ml gave the best results in decreasing the total *Vibrio* and the survival rate of vanamei shrimp compared to the probiotic *Bacillus* and *Pseudomonas* with a density of 10⁶ cells/ml (Satyantini *et al.*, 2019).

The salinity in the pond is influenced by the salinity of the water source and evaporation. Salinity plays a role in shrimp's osmoregulation and the moulting process. In ponds 1,2,3, and 4, the salinity values are the same. The resulting salinity value is still in the category suitable for shrimp growth (Arsad *et al.*, 2017).

The pH value during the maintenance process is still optimal from 7.6 to 8.2. The pH value should not fluctuate high because it will cause shrimp stress, decrease appetite, and affect shrimp immunity (Multazam & Hasanuddin, 2017). A low water pH value will result in shrimp death, while a high water pH can cause low growth (Salam *et al.*, 2019).

The relationship between dissolved oxygen and shrimp is continuous and influences each other. Dissolved oxygen levels in ponds 1,2,3, and 4 are in the same optimum range. Dissolved oxygen levels fluctuate daily and seasonally, which affects the photosynthetic activity and respiration of aquatic biota. The stability of the value of dissolved oxygen in the waters also affects the growth of shrimp in high density, both from the needs of the shrimp themselves and the microorganisms that live in them. The microorganisms in question are probiotic bacteria and plankton (Effendi, 2003).

The temperature values in ponds 1,2,3 and 4 are optimal. The optimal temperature for shrimp ranges from 26-34 °C (Suwarsih *et al.*, 2016). The high water temperature causes the oxygen in the water to evaporate, resulting in the shrimp larvae being deprived of oxygen. The low water temperature will cause the shrimp's appetite (Adipu, 2019). If the water temperature is below 24 °C, it is better to check the desire using anco control.

An increase in water temperature of 10 °C will cause an increase in oxygen consumption by aquatic organisms to be two to three times. An increase in temperature will also be followed by a decrease in dissolved oxygen value so that it cannot meet the oxygen demand in the waters. Stirring with the wheel is continuously carried out so that higher temperature fluctuations do not occur (Effendi, 2003).

Probiotics that are fermented first will cause an increase

in the individual bacteria contained so that the work of bacteria will be more effective. The density of bacteria in probiotic B was lower, but the fermentation process carried out was able to increase the yield of bacteria. Table 3 shows that ponds 3 and 4 using probiotic B had lower ammonia content than ponds 1 and 2 using probiotic A. This indicates that the bacteria in probiotic B decompose organic matter and suppress ammonia content.

Although probiotic B had a lower log unit rating than probiotic A, it first proceeded with fermentation. The log unit rank of bacterial colony density increased, and probiotic B had a higher bacterial density than probiotic A. At the media preparation stage, probiotic A and probiotic B are given one mg-l every day continuously for three days, and during the maintenance, the period is offered five times with a dose of 1 mg-l (according to the product's recommended use). But at DOC 75, probiotics are given every two days at a quantity of 2 mg-l until harvest time.

CONCLUSION

The content of more probiotic bacterial variants indirectly results in higher productivity. The bacterial composition will remodel organic matter and feed into compounds that are easy to digest. Probiotic B also has a better effect on water quality.

The content of more bacteria inside, such as in probiotic B, resulted in a better range of air-water quality for the survival rate of vanamei shrimp and FCR value, resulting in higher productivity. The probiotic fermentation process will increase the density of the bacteria inside, making it more effective to use.

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