**EFFECTIVENESS OF DIFFERENT OF PROBIOTIC APPLICATION ON VANNAMEI SHRIMP PRODUCTIVITY**

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**Abstract.**

Vannamei is a superior commodity of aquaculture in Indonesia. However, production of vanamei only reached 91.5% of the revised target of 450,000 tons. Several factors are the cause of the 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 aplication probiotic to control pathogenic microorganisms is better influenced by the type of bacteria used. This research was conducted to examine the effectiveness use of two different types probiotics with defferent bacteria inside on the productivity of vannamei shrimp. The pond plots used were coated with Low Density Poly Etylene (LDPE) with a plot area of 2200 m2. Stocking density of 250 fry m-2 of each. During rearing, the vannamei shrimp were fed pellet containing 30-40% protein, 5% fat, 4% fiber, and 11% water content previously fermented with probiotics A and B. The results showed that the use of type B probiotics is better productivity than type A.

Keywords: *financial analysis, probiotics, vannamei productivity*

1. **Introduction**

Vannamei shrimp is an important economic commodity because of its fast growth, high survival rate, not susceptible to disease and widely cultivated throughout the world (Li et al., 2016 in Santanumurti et.al., 2019). Vannamei shrimp contribute 40% of all fishery products and foreign exchange earnings (Hadie and Hadie, 2017).

Intensive and super-intensive shrimp farming activities using high density and feeding resulted in an increase in feed residues and shrimp feces, which resulted in a decrease in water quality, such as an increase in organic matter, ammonia and pathogenic organisms. This decrease in environmental quality causes shrimp to become stressed and susceptible to disease. One of the diseases that often attack shrimp is Vibriosis, which is caused by bacteria from the genus Vibrio. Vannamei shrimp that are 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 vanamei is a better choice than the use of antibiotics for treatment. In addition to antibiotics it can have a resistant effect on bacteria in cultured animals to apply a regulation on zero tolerant antibiotic residues has been established (Silaban, 2007 in Rume et al., 2012). Probiotics protect vanamei by producing chemical compounds that are bactericidal and bacteriostatic against other bacterial populations.

The effectiveness of using probiotic to control pathogenic microorganisms is influenced by the type of bacteria used. The life of bacteria is influenced by the environment. The population of bacteria in the environment with different nutrient and physico-chemical content will generally be different (Suprapto, 2008 in 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 and reproduce and carry out their roles (Muliani et al., 2008).

Study of the product to determine of the probiotic bacteria used with the needs of the pond to be carried out with the desired purpose of using the probiotic itself. One of the probiotic products known to be good enough to improve shrimp health was tested to see the composition of probiotic bacteria. The results of bacteria in probiotics were studied against other probiotic products to see the water quality and production of shrimp ponds. That were affected by the two probiotic products.

1. **Materials and Methode**

This research was carried out from August to December 2020 at the pond of PT Loligo, Sidoarjo, East Java. There have several ponds, but the ones used for research are ponds 1,2,3 and 4. The pond plots used were coated with Low Density Poly Etylene (LDPE) with a plot area of 2200 m2. The treatment tested was the application of probiotics with different bacteria inside on the productivity of vannamei shrimp.

* 1. *Pond preparation*

The pond used is . Low Density Poly Etylene (LDPE). The number of ponds is 4, with dimensions of 44 x 50 m2 and 2 mdepth or 2200 m2 pond-1. Overall pond preparation takes ± 1 month, after the previous cycle used for shrimp farming. The stages of pond preparation carried out are pond drying, liming, fertilizing, layer repair and pond construction. Clean the pond from dirt and crop residues. Leveling the soil base and resetting the slope towards the central drain. Spreading dolomit (CaMg (CO3)2) dan kapur tohor (CaO) at a dose of 2 kg m-2 until for 3 days. On the day fourth, organic fertilizer is spread a dose of 0.5 kg m-2.

* 1. *Water treatment*

Water treatment is done after application of fertilizer. Water is transferred by pump from the reservoir to the maintenance 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 2 days, the chlorine content was tested. After the water is neutral, CaCO3 is added at a dose of 50 mg-l. The next day, 1000 mg l-1 of probiotic A and B was added for 3 days in the afternoon.

* 1. *Stock density*

The vannemei fry were stocked with SPF (Specific Pathogen Free) certified PL 11 stage. The fry were spread in the morning and previously acclimatized for 30 minutes. The stocking density of the fry is 250 m-2 (550.000 pond-1).

* 1. *Feed Management*

Feeding is based on the blind feeding program until the age of 30 days and the anco feeding program after shrimp can trapped by the sampling nets. The feed used is artificial feed which has 3 types, namely fine crumble, crumble and pellet. Fine crumble feed was used at the beginning of the rearing period until DOC 30. Then it was replaced with crumble feed. Each feed type change, the previous feed was mixed with substitute feed with a ratio at age 29 of 75%: 25%, age 30 50%: 50%, and age 31 25%: 75%. 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 Convertion Ratio) was calculated after the vanamei was harvested. This is to determine the effectiveness of the feed and the effect of the probiotics applied.

* 1. *Water Management*

Siphoning and water changes are intended to remove uneaten feed and shrimp or plankton waste that settles at the bottom of the pond and change the water every day at 09.00 AM. Addition to the original water level with sterilized reservoir water. To improve water quality, two different probiotic bacteria inside, probiotic A and probiotic B, were used. In ponds 1 and 2, probiotic A were used, while in ponds 3 and 4 were probiotic B. Probiotic A was given at a dose of 1 liter ha-1. Probiotic A is directly applied without having to be fermented first. For ease of use diluted with water beforehand. Probiotic B must be fermented and multiplied before use. One liter of probiotic B plus 1 kg of pellet powder and sea water to 100 liters, then aerated for 24 hours. Probiotic B application at 05.00 PM. Probiotics A and B probiotics were applied in the morning once every 5 days. 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.

Table 1. Composition, application methode and density of probiotic A and probiotic B

|  |  |  |
| --- | --- | --- |
|  | Probiotik A | Probiotik B |
| Bacteria composition | *Pseudomonas* spp | *Pseudomonas putida* |
|  | *Pseudomonas pseudoalcaligenes* | *Aspergillus niger* |
|  | *Bacillus* spp | *Bacillus subtilis* |
|  | *Acinetobacter calcoaceticus* | *B. lichenformis* |
|  |  | *B. coagulans* |
|  |  | *B. megaterium* |
|  |  | *B. polymixa* |
| Application methode | Non fermented | Fermented |
| Density | 3,0×108 CFU ml-1 | 106 CFU ml-1 |

* 1. *Growth measurement*

Growth was carried out by sampling at DOC 35, and thereafter every 7 days until harvest to determine the growth and condition of shrimp. Sampling using nets, carried out every 10 days. Sampling was carried out in the morning. The calculated data includes ABW (Average Body Weight), population, biomass.

* 1. *Harvest and Post Harvest*

Harvest was obtained at DOC 90, in total by using nets. The harvest is sorted to separate the size of the shrimp.

1. **Result and Discussion**

Figure 1. The average body weight of shrimp at 9 times of sampling.

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 90, samples were counted at harvest. Until the 7 th sampling, 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 90 it can be seen that the ABW in pond 3 and 4 is higher than pond 1 and 2 . Organic matter will increase with the age of shrimp rearing, which comes from the remaining feed and the rest of the metabolism of shrimp and 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, to produce enzymes in large quantities, to fight pathogens by inhibiting their growth and to compete competitively. Bacillus sp. also have thick cell walls so that they live longer, are resistant to dehydration, and are resistant to temperatures up to 60°C (Decamp and David, 2005). *B. subtilis* produces the enzyme bacitracin. *B. lichenformis B. coagulans*, *B. megaterium. B. polimyxa* produces the enzyme polymyxin. stated that Bacillus lichenformis bacteria can produce several extracellular enzymes, namely amylase, amino peptidase, metal proteases, lactamase, endo-N-acetylglucoaminidase, and lipase. These bacteria are also resistant to alkaline environments (Soeka *et a*l., 2011).

In cultivation, there are two factors that must be considered to create a stable microorganism community, namely 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. While the stochastic factor is the opportunity for microorganisms to enter at the right time and environment in order to live. Probiotic A has a density of 3.0×106 CFU/ml, while probiotic B has a density of at least 106CFU/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 it is certain that 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 3 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 every day (stochastic). These two factors are the most likely to influence the effective work of probiotics outside of the bacterial species that affect it. High value of feed efficiency, indicated the shrimp response better to feed so it was indicated by the growth of the shrimp (Hariyadi *et al.,*2005 in Anwar. S , *et.al.* 2016).

The increase in ABW of shrimp was also influenced by CaMg (CO3)2 and CaO which were applied during preparation and cultivation of shrimp. Dolomite and quicklime lime does not directly affect the growth of shrimp, but can meet the needs of calcium and minerals when moulting and 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 (Yulihartini et.al. 2016 in Yunus, *et.al.,* 2020).

Table 2. Pond Productivity

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 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 |

Table 2 shows that there are differences in Survival Rate values ​​in ponds 1, 2, 3 and 4. The feed conversion ratio is one of the parameters that can be used as a measure of 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 use of feed in increasing the weight of the cultured shrimp Cousin et al., (1996) in Zainuddin *et al*. (2019).

The presence of microorganisms in the vaname shrimp's digestive tract allows it to help the process of digestion of food that enters the body. According to Leano et al. (2005) in Zainuddin et al. (2019). Novitasari (2017) stated that mixing feed with Bacillus sp bacteria can increase the growth of vannamei shrimp. The addition of Bacillus sp into the feed with the right amount can suppress FCR.

The addition of probiotics can reduce the population of Vibrio sp. The same results are also shown by this study. This is in accordance with the opinion of Soundarapandian *et al*., (2010) and Yudiati *et al.,* (2006) in Yudiati *et al*. (2010) who reported that probiotic bacteria derived from shrimp rearing culture media were potential and can suppress the growth of pathogenic bacteria including *Vibrio* sp. Ammonia-degrading bacteria *Nitrosomonas eutorpha* and *Nitrobacter winogradsky* and organic matter-decomposing bacteria *Paracoccus pantotrophus* and *Bacillus* sp when applied simultaneously were able to increase the survival rate by 6-7% but did not increase the growth of white shrimp (*L. vannamei*) tocolans. Giving probiotics is also able to reduce the total population of Vibrio, total bacteria.

The bacteria *Pseudomonas putida* used have not been able to provide a consistent and significant effect on BOT, NO2-N, PO4-P, and NH4-N, but have a significant effect on H2S at the end of the study, so it has an impact 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 vaname shrimp compared to the probiotic Bacillus and Pseudomonas with a density of 106 cells/ml. (Satyantini et.al.,2019).

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameters | Pond 1 | Pond 2 | Pond 3 | Pond 4 |
| Salinitas (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 |
| Suhu (oC) | 29.1 - 30.5 | 29.2 – 31.8 | 29.2 – 31.9 | 29.4 – 32.1 |
| Ammonia | 0 - 0.5 | 0 – 0.5 | 0 - 0.25 | 0 – 0.25 |
| nitrit | 0 – 0.5 | 0 – 0.5 | 0 – 0.25 | 0 – 0.25 |

The salinity in the pond is influenced by the salinity of the water source and evaporation. Salinity plays a role in the osmoregulation of shrimp and also the molting 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 and decrease appetite and affect shrimp immunity (Multazam & Hasanuddin, 2017). A low water pH value will result in the death of shrimp while a high water pH can cause low shrimp growth (Salam *et al*., 2019).

The relationship between dissolved oxygen and shrimp is continuous and influences each other. Dissolved oxygen levels in pond 1, 2, 3 and 4 are in the same range, and it the 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 in the optimal range. The optimal temperature for shrimp ranges from 26-34℃ (Suwarsih *et al*., 2016). The high water temperature causes the oxygen in the water to evaporate which results in the shrimp larvae being deprived of oxygen. The low water temperature will cause the shrimp's appetite to decrease (Adipu, 2019). If the water temperature is below 24ºC, it is better to check the appetite 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 the value of dissolved oxygen 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 f bacteria in probiotic B was lower, but the fermentation process carried out was able to increase the work of bacteria. Seen at the lower values ​​of ammonia and nitrite in ponds 3 and 4. Adipu (2019) also stated that the addition of carbohydrate sources had a better effect because bacteria would use them. which results in the inhibition of the inorganic nitrogen formation process so that it will reduce the amount of inorganic nitrogen in the water column.

**4. Conclusion**

4.1. The content of more probiotic bacterial variants indirectly results in a higher Survival Rate. Bacterial variants will remodel organic matter and feed so that they become compounds that are easy to digest. Probiotic B also has a better effect on water quality.

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

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