Effects of different times of probiotic additions on floc abundance and growth of white leg shrimp (*Litopenaeus vannamei*): Laboratory scale cultivation

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Abstract. Water quality management for aquaculture is very important because water is a living medium for aquaculture organisms. One of the technological products that use microbes to create a better environment by breaking down organic matter is probiotics. This study aims to determine the effects of different probiotic administration times on water quality in white leg shrimp culture at a laboratory scale. Juveniles of white leg shrimp with PL27 size were reared for 45 days and given four treatments with probiotics at different times. The result showed that the administration of probiotics with a short period interval had a significant effect on floc volume, absolute growth, survival rate, concentrations of ammonia, nitrate, and nitrite. Provision of probiotics with a short time span tended to reduce the concentration of ammonia, nitrate and nitrite. The difference in time of giving probiotics to bio-floc media increased the growth of white leg shrimp, improved water quality, and reduced the concentration of ammonia (NH₃), nitrate (NO₃⁻), and nitrite (NO₂⁻).

1. Introduction

Bio-floc technology (BFT) is an alternative to solve the waste problem in intensive cultivation. This technology is very beneficial because besides being able to reduce inorganic nitrogen waste from leftover feed and manure, it can also provide additional protein feed for farm animals so that it increases growth and feed efficiency. Thus, this technology can be used as a solution in improving bio-floc technology by adding organic carbohydrates to maintenance medium in order to increase the C/N ratio and stimulate the growth of bacteria where it can assimilate inorganic nitrogen into bacterial biomass [1]. Probiotics contain mostly microorganisms *Lactobacillus*, *Nitrosomonas* and *Nitrobacter* which can increase decomposition of waste and can improve water quality [2]. The application of probiotics in aquatic environments can increase shrimp immune response to disease, improving their digestive system, improving water quality because it can convert toxic compounds into non-toxic such as ammonia and nitrite compounds through the nitrification process [3], increasing shrimp growth rate, and increasing survival rate of shrimp thus increasing production [3], [4].

Management of water quality for aquaculture is very important because water is a living medium for aquaculture organisms [5], [6]. One way to overcome water pollution due to organic waste is to use technology that can utilize microorganisms to remodel organic matter. One of the technological
products that use microbes to create a better environment by breaking down organic matter is probiotics [7].

The application of probiotics has been carried out by some researchers. They reported that probiotics have a fairly good effect on the water quality condition of white leg shrimp culture media [8], [9]. Thus, the purpose of this study was to determine the effect of different probiotic administration times on water quality in white leg shrimp culture at a laboratory scale.

2. Material and methods

2.1 Research preparation
This research was conducted for 45 days at the Wet Laboratory of the Faculty of Fisheries and Marine Affairs, Khairun University, Kastela Village, Ternate, North Maluku, Indonesia. The size of juveniles of white leg shrimp (Litopenaeus vannamei) which is used in this research was PL27. Seawater and fresh water are used after the filtering and sterilization process. In this study, probiotic (EM4) was used for treatment. To maintain the C/N ratio in the water, molasses was given according to container volume [10]. Others equipment needed, among other, were basins, fibres tube, blowers, aerators and Imhoff cones.

2.2 Research design
The preparation of containers in this study includes cleaning of all the equipment to be used as well as labelling each container. There were 12 units of containers with a size of 30 litters. Each container is given aeration for the oxygen supply needed by shrimp juveniles. Before being stocked into containers, the juveniles were first acclimatized for 3 days. Juvenile shrimp with a size of PL27 were stocked in each container according to the number of different stocking densities as treatment. The treatments were consisting of treatment A, every 5 days (9 times of probiotic administration during the study); treatment B, once every 10 days (10 times of probiotic administration during the study); treatment C, once every 15 days (15 times the administration of probiotics during the study); and treatment D, as the control (without giving probiotics during the study). The placement of each experimental unit was done randomly.

2.3 Parameter measurement
The parameters measured in this study were floc abundance, growth (gr), survival rate (SR), concentration of ammonia, nitrate and nitrite. In addition to these parameters, measurements of water quality such as temperature, salinity, pH, DO were carried out in situ.

Measurement of floc abundance was carried out once a week. Measurements were made using an Imhoff cone. The floc measurement used the method modified by others researcher [3]. Absolute weight growth and survival rate were carried out by using the formula [11]. Meanwhile, ammonia, nitrate and nitrite were analysed by using the methods of APHA [12].

2.4 Data analysis
All data were analysed using variance (ANOVA) at a significance level of 95%. The LSD test is used if the treatments have a significant effect [13]. Meanwhile, water quality parameter data were analysed descriptively and presented in table form.

3. Results

3.1 Absolute weight growth
The absolute weight growth varied in each treatment. The absolute weight growth was better in treatment A (4.26 gr) followed by treatment B, C and D. The results of observations during the study are shown in Figure 1.
3.2. Survival rate (SR)

The survival rate (SR) of white leg shrimp during the study seemed to vary between all treatments. The highest survival rate was found in treatment D followed by treatment A, B and treatment C. The survival rate observations are presented in Figure 2 below.

![Figure 2. Survival rate (SR) of white leg shrimp during the study](image_url)

The results of analysis of variance showed that the administration of probiotics had a significant effect on the survival rate of white leg shrimp. The LSD test showed that treatment D had significant effects.

3.3 Floc volume
Floc volume during the study between treatments were seemed to vary. The highest floc volume was found in treatment A followed by treatment B and treatment C. The results of the measurement of floc volume during the study are shown in Figure 3 below.

The results of analysis of variance showed that the difference in the timing of probiotic administration had a very significant effect on the volume floc. The LSD test showed that treatment A showed a very significant treatment.

![Figure 3. Volume floc during the study period](image1)

**3.4 Ammonia (NH₃)**

The results of the observation of ammonia concentration during the study showed variations between the four treatments. At the beginning of the maintenance of the four treatments showed a tendency to decrease ammonia until the third and fifth weeks. Then in the sixth week only treatment A showed a trend of decreasing ammonia. While in treatment B, C and treatment D had a tendency to increase. The results of ammonia measurements are presented in Figure 4 below.

![Figure 4. NH₃ concentration during the study](image2)

The results of analysis of variances showed that the difference in the timing of probiotic administrations had a very significant effect on ammonia (NH₃). The LSD test showed that treatment A showed a very significant treatment.
3.5 Nitrate (NO$_3$)

The concentration of nitrite (NO$_3$) during the study in each treatment varied greatly. However, in treatment A, it seemed to tend to decrease compared to other treatments. The results of nitrate measurements during the study are shown in Figure 5 below.

The results of analysis of variance showed that the difference in the timing of probiotic administration had a very significant effect on nitrate (NO$_3$). The LSD test showed that treatment A showed a very significant treatment.

![Figure 5. NO$_3$ concentration during the study](image5.jpg)

3.6 Nitrite (NO$_2$)

The concentration of nitrite (NO$_2$) in the four treatments showed fluctuations every week. However, treatment A was relatively constant compared to the other treatments. The results of the measurement of nitrite (NO$_2$) during the study are shown in Figure 6 below.

![Figure 6. NO$_2$ concentration during the study](image6.jpg)

The results of analysis of variance showed that the difference in the timing of probiotic administration had a very significant effect on nitrate (NO$_2$). The LSD test showed that treatment A showed a very significant treatment.
3.7 Water quality

Water quality parameters such as temperature, salinity, pH and DO during the maintenance period were relatively constant. The results of water quality measurements measured during the study period are shown in Table 1 below. The range of all water quality parameters is still in the optimal for the growth of white leg shrimp.

| No | Treatments | Parameters |
|----|-------------|------------|
|    |             | Salinities (ppt) | Temperature (°C) | pH | DO (mg/L) |
| 1  | A           | 24-25        | 25-28            | 7.0 | 2.9-4   |
| 2  | B           | 24-25        | 25-28            | 7.0 | 2.9-4   |
| 3  | C           | 24-25        | 25-28            | 7.0 | 2.9-4   |
| 4  | D           | 24-25        | 25-28            | 7.0 | 2.9-4   |

4. Discussion

The absolute weight growth of white leg shrimp in each treatment varied greatly and increased from the beginning to the end of the week (Figure 1). The gain of shrimp weight was caused by optimal feeding and addition of probiotics as additional feed (floc). From the three treatments that were tried, it appears that treatment with a relatively short time range of giving probiotics had a significant effect on the growth of white leg shrimp. Some studies showed that shrimp growth increased due to the effect of adding probiotics in the rearing media so that bacteria in probiotics functioned to improve water quality and also had effect to improve digestive system of white leg shrimp [14]. Meanwhile, stated that the provision of probiotics in bio-floc technology would increase the nutritional content such as protein and fat in the flock which could increase shrimp growth [7], [15]. Probiotics that enter the body of white leg shrimp are very useful for digestion where bacteria were able to secrete digestive enzymes such as proteases and amylase so that it has an impact on optimal digestibility of feed [16], [17]. The types of bacteria capable in secreting digestive enzymes are such as the genus Bacillus sp. These bacteria are able to produce protease and amylase enzymes so that the digestibility of white leg shrimp increases. The presence of probiotics in the shrimp’s digestive tract can also increase feed absorption and suppress the number of pathogens in the digestive tract [15], [18].

The highest survival rate (SR) was seen in the control treatment (100%), which was higher than the other treatments (Figure 2). This phenomenon was closely related to the difference in stocking density in each treatment [10], [19]. Higher density of shrimp tended to lower survival rate that was related to oxygen consumption, space for movement and the absence of competition between individuals [10], [20], [21]. However, the administration of probiotics significantly affected survival rate in all four treatments, where treatment A showed an increase of 73.33%, treatment B (68.33%), and treatment C (68.00%) were still in the ideal category in cultivation. shrimp [22], [23], [24].

Floc volume is the amounts of suspended solids over a certain period of time in an inverted conical container [25]. The high value of floc volume in bio-floc treatment showed that bacteria in rearing ponds could form flocs which could then be used by shrimp as additional feed for growth and could reduce the amount of feed given [26]. According to [7] the maximum floc volume was 150 ml/L or 15% of the water volume. The high volume of floc in treatment A showed a better performance of bacteria compared to other treatments [27]. It was further explained that the floc-forming bacteria would break down organic matter (protein, carbohydrates, and fat) from feed residues, shrimp droppings and dead bodies in the pond. One of the limiting factors that affect the volume of the floc is the availability of sufficient dissolved oxygen levels in the water [28]. Ammonia will be synthesized into protein cells by several types of bacteria, and some are oxidized by nitrifying bacteria to nitrite by Nitrosomonas bacteria and then from nitrite to nitrate by Nitrobacteria [7], [26]. The volume of floc in this study is still optimal for white leg shrimp culture [29].

The concentration of ammonia (NH₃) during the maintenance period tended to decrease in the administration of probiotics in a short time span (treatment A), it is in contrast to other treatments,
especially the control treatment. It appeared that the performance of bacteria was more efficient in a short time span [30], [31]. Decomposing bacteria such as Nitrosomonas broke down ammonia into nitrite more efficiently by administering probiotics. According to [27] the addition of probiotics into the maintenance container was able to suppress the ammonia level in the water so that it did not experience a significant increase. The range of ammonia concentration during the rearing period was still in the optimal category for white leg shrimp culture with a value of <1.22 mg/L [32].

The concentrations of nitrate ($\text{NO}_3$) and nitrite ($\text{NO}_2$) during the rearing period varied in each treatment (Figures 4 and 5). Concentration that significantly affects water quality was treatment with a short time span. It seemed that the performance of bacteria was more efficient where the number of bacteria was increasing in the floc, so that the decomposition of ammonia into nitrate was faster and more effective [33]. The concentration values of these two compounds were still good for white leg shrimp cultivation [2], [10]. The range of fluctuations in water quality parameters such as temperature, salinity, pH and DO during maintenance was considered optimal [2], [10], [34], [35].

5. Conclusion
From this research, it was found that the difference in the time period of giving probiotics to bio-floc media increased the growth of white leg shrimp, improved water quality, and reduced the concentration of ammonia ($\text{NH}_3$), nitrate ($\text{NO}_3$), and nitrite ($\text{NO}_2$).

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