The Effect of Probiotic Doses in Biofloc Growth on Hematological and Histological Status of Catfish

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Abstract

Biofloc technology is one of the promising methods to overcome water quality problems in fish farming. This technology uses an environmentally friendly approach and does not harm cultivated organisms. The success of biofloc technology depends on the accuracy of determining probiotics and doses in the treatment. This study used petrofish commercial probiotics containing *Lactobacillus heterochiochii*, *Bacillus* sp., *Nitrosomonas* sp., and *Saccharomyces cerevisiae*, with a bacterial density of 1.2 x 106 CFU.mL^{-1}. This study aimed to determine the suitable doses of probiotics for growing biofloc. Biofloc has a high content of protein. In the biofloc process, fish will consume the floc. It led to hematology status and intestine histology alteration that caused effects on fish growth. The method used in this study was an experimental method with a completely randomized design with four treatments. Each treatment will be repeated three times. The variable test was a difference of doses of probiotic P0 (0 L.L^{-1} water), P1 (1 L.L^{-1} water), P2 (1.5 L.L^{-1} water), and P3 (2 L.L^{-1} water). The observations on the value of hematological status showed erythrocytes in the P2 treatment was 2.9 x 1012.L^{-1} and P3 was 2.5 x 1012.L^{-1}. It was categorized as a normal condition, while P0 and P1 (2.0 x 1012.L^{-1}) were below normal values. The hemoglobin values of P2 (9.6 g.dL^{-1}) and P3 (9.0 g.dL^{-1}) were in accordance with normal values, while P0 (4.9 g.dL^{-1}) and P1 (6.1 g.dL^{-1}) were below normal. The hematocrit value in P2 was under the normal limit of 39%, while the treatments P0, P1, and P3 were below the normal limit. The leucocyte values in all treatments (P0, P1, P2, and P3) were above the normal (high) value, influenced by high stocking density. The best intestinal villi length was found in P2, which is 2431.42 µm; the villi width is 631.90 µm. Based on the observations on all variables, it can be stated that the probiotic doses have significant biofloc growth and hematological and histological intestine status. Furthermore, the (P2) treatment with 1.5 L.L^{-1} water effectively affected the growth of catfish (*Clarias* sp).

Keywords: Biofloc, Catfish, Hematology, Histology, Probiotic Petrifish.

INTRODUCTION

Areas with low rainfall and a lack of freshwater resources can be affected by the lack of potential land for aquaculture. Therefore, adopting a suitable aquaculture method to increase production is necessary. One of the methods in aquaculture with environmentally friendly essentials is biofloc technology [1]. The basic principle of biofloc technology is to produce nitrogen cycles in a stagnant state in aquaculture systems by increasing the growth of probiotic bacteria. The bacterial population will convert inorganic nitrogen substances into high-protein biofloc that fish can utilize as natural food [2]. Bioflocs can reduce the cost of fish farming activities by providing additional protein feed through mixing bacteria, feed residues, feces, and other impurities and plankton in strong aeration, and also efficiency in water use [3]. The protein in the feed given to fish is only utilized about 25% for the growth and life process, while the remaining 75% will be released into their habitat [4].

This condition may be affected the water quality decrease. Therefore, using probiotic bacteria in the cultivation medium is necessary to convert feces and ammonia into living flocs as a natural food with high protein content. The fish consumed biofloc, the protein which will contain in the body of fish and influences the hematological status and the absorption process of nutrients in the fish intestine to be high. The suitable probiotic dose will provide the number of probiotic bacteria to fulfill the needs of the cultivation media with the support of a good C/N ratio and high dissolved oxygen. It will affect water quality and biofloc growth [5]. In order to facilitate the increase in production, many types of probiotics have been produced and sold freely on the market.

One is petrofish commercial probiotics containing *Lactobacillus heterochiochii, Bacillus* sp.*, Nitrosobacter,* and *Saccharomyces cerevisiae* with a total bacterial content of 2 x 10^4 CFU.mL^{-1} [6]. The petrofish probiotic will be tested with different doses in cultivating catfish *Clarias* sp. with a biofloc system. The carbon source was pellet and sugar at a 16 C/N ratio. The stocking

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density was 1000 individuals.m^{-3}, referring to the research of [7]. The purpose of the study was to determine the suitable dose for growing biofloc that can affect the hematological and histological intestine status and also the growth of *Clarias* sp.

MATERIAL AND METHODS

This research was conducted from May 20 to August 01, 2022, in the outdoor laboratory Department of Aquaculture, Faculty of Agriculture and Animal Husbandry, University of Muhammadiyah Malang, located in Tegalgondo Village, Karangploso Sub District, Malang Regency, East Java, Indonesia. The method used in this research was experimental with a completely randomized design (four treatments with three-time replication).

Fish Preparation

The fish used in this study was catfish (*Clarias* sp.) with a size of 7 cm and a weight of ± 5 grams.individu^{-1}. In this study, the stocking density used was 1000 fish.m^{-3} [8]. Fish are fed with 30% of feed content. Fish feeding was carried out according to 5% by weight of the biomass of the test fish.

Water Quality Monitoring

Several water quality parameters tested in this study include pH, temperature, DO, Ammonia, and nitrite. DO, pH, and temperature observations were carried out daily using a DO meter, pH meter, and thermometer. While testing for ammonia and nitrate was carried out weekly using a test kit and a spectrophotometer with the brand Sera.

Hematology Assay

Hematology testing was carried out twice, at the beginning and the last of the research. Fish blood was taken using a syringe with a capacity of 1 mL. The syringe used to draw blood is moistened with an anticoagulant solution such as EDTA to prevent blood clots from forming in the syringe [7]. Blood samples were taken by injecting a syringe in the linea lateralis at an angle of 45°. It is intended to make it easier for blood to enter the syringe tube. After obtaining the blood sample, it was put into a microtube with a capacity of 1.5 mL. The blood samples were tested at the Animal Health Clinic Laboratory in Tidar, Malang City. Several parameters were observed, including the number of erythrocytes, leukocytes, hemoglobin, hematocrit, and differential leukocytes (neutrophils, lymphocytes, and monocytes).

Intestinal Histopathology Assay

Histological procedures were carried out according to the method of Santos *et al.* [8] with a few modifications. The fish were dissected, and the intestinal organs were taken and then fixed in 10% formalin solution for 70 hours. After that, the intestinal samples were hydrated in graded ethanol solution and cleaned using xylol solution. Next, the sample is embedded in paraffin at a temperature of 60°C. The preparations were then cut using a microtome with a thickness of 5 µm. Then the preparations were stained with a hematoxylin solution and eosin with a bacterial density of 2x 10^{4} CFU.mL^{-1}. The doses used in this study were 0 mL.L^{-1} as a control (P0), 1000 mL.L^{-1} (P1), 1500 mL.L^{-1} (P2), and a dose of 2000 mL.L^{-1} (P3).

Data Analysis

Quantitative data from observations and processing of hematological parameters, intestinal histology, and water quality were analyzed using one-way ANOVA. The result showed a significant difference between treatments, so it was continued with the Duncan Multiply Range Test (DMRT) at the level of significant $P< 0.01$ using the SPSS 26 program.

RESULT AND DISCUSSION

Water Quality

Water is the habitat of all aquatic organisms. Water quality significantly affects the survival of aquatic organisms. One of the causes of failure in fish farming is poor water quality. Biofloc technology has become a promising method for managing good water quality. The success of biofloc depends on the content of probiotic bacteria and other supporting factors in the cultivation medium. Data from water quality measurements during the study were analyzed and averaged for each treatment. Then the results were compared with normal values tolerated by catfish according to the literature. The results of Average water quality during the study are presented in Table 1.

In accordance with the data in Table 1, the total density of probiotic bacteria in the P2 treatment until the end of the study showed more than in other treatments. In the P2 treatment, biofloc volume was to be higher at 128 mL.L^{-1}, and the levels of ammonia and nitrate tended to be low and tolerated by catfish. Moreover, DO and pH still show optimal levels for catfish growth. The DO obtained in the study exceeded the minimum standard in catfish farming, which was more than 3 mg.L^{-1}.

Table 1. Average Water Quality Values during the Study

Parameter	Normal Standard [5,9,10]	Average value during the study			
		P0 (0 $L.L^{-1}$)	P1 (1 $L.L^{-1}$)	P2 (1.5 $L.L^{-1}$)	P3 (2 $L.L^{-1}$)
Temperature	27-30	24.88	24.88	24.88	24.75
DO ($mg.L^{-1}$)	≥3	3.86	3.86	4.24	3.51
pH	6.5-8	7.3	7.29	7.29	7.25
Ammonia ($mg.L^{-1}$)	≤0.5	0.72	0.66	0.33	0.57
Nitrate ($mg.L^{-1}$)	<20	1.43	1.83	1.56	2.19
Total bacteria (10^5)	$CFU.mL^{-1}$	-	0.20 ± 0.07^a	1.10 ± 0.87^b	0.24 ± 0.02^a
Biofloc Volume (mL)	50-500	-	72.22 ± 5.09^a	128.0 ± 2.65^c	91.89 ± 4.07^b

DO is one of the essential factors in fish rearing. In a culture system, DO is used by cultured organisms for the respiration process. Besides that, microorganisms often use DO to break down organic materials and metabolic wastes. The source of DO in the waters comes from direct diffusion from the environment and can be produced by phytoplankton through photosynthesis [11]. pH is one of the limiting factors in the life of aquatic organisms. The pH value indicates the concentration of hydrogen ions in water [12]. The optimal range of pH values for catfish growth is between 6.5-8. Low pH values can interfere with the metabolism of aquatic organisms. Several studies have shown that aquatic organisms will produce excess plasma glucose in acidic water conditions [13].

The temperature value at the time of the study was lower than the optimal range for catfish growth. The low value obtained during the study was influenced by the time and geographical location of the study. The research was carried out from June to August and precisely at the University of Muhammadiyah Malang, located in the highlands (440-667 m asl), where the rainfall was relatively from July to August high during the study. Catfish can grow optimally at temperatures ranging from 27-30°C [14].

The average value of ammonia in the study exceeded the threshold where the tolerance for ammonia for catfish growth was 5 $mg.L^{-1}$, and the nitrate value for catfish growth was still around 20 $mg.L^{-1}$. The high value of ammonia can result in the death of fish. It is because ammonia has toxic properties in cultured fish [15]. The high ammonia value in the study could be caused by the high metabolic residues in the cultivation system that microorganisms had not decomposed. In water, nitrate functions as a microalgae nutrient. Microalgae will utilize nitrate for the growth and development process [16].

The floc formed in the aquaculture system is still considered optimal. The optimal value of formation ranges from 50-500 mL. Floc is a heterotrophic bacterial biomass collection that will later form aggregates. Floc is composed of bacteria and other microorganisms, such as microalgae and zooplankton [17].

Hematology

The results of hematological observations during the study which were presented in Table 2. The values of erythrocytes, hemoglobin, and hematocrit in the P2 and P3 treatments were in the normal category according to the minimum standard. Meanwhile, P1 and P0 were below the normal standard. The normal values of erythrocytes, hemoglobin, and hematocrit in P2 and P3 treatments can facilitate the distribution of nutrients to all body tissues. The function of erythrocytes and hemoglobin is to bind oxygen and nutrients to be circulated throughout the body's tissues for metabolic and growth processes in addition to circulating nutrients and oxygen. Hemoglobin also transports the waste products of metabolic processes back to the kidneys and is excreted in the form of feces and urine [18].

Table 2. Average Value of Hematology Parameter during the Study

Parameter	Unit	Standard [18]	Before Biofloc Treatment (P0, P1, P2, P3)	After Biofloc Treatment			
				P0	P1	P2	P3
Hemoglobin	$g.dL^{-1}$	6.30 - 14.24	6.70	4.80	5.30	9.60	6.60
Hematocrit	%	37.00 - 45.00	39.00	30.10	21.20	39.40	36.20
Erythrocytes	x $10^{12}.L^{-1}$	2.20 - 6.20	2.80	2.10	1.70	2.70	2.40
Leukocytes	x $10^{12}.L^{-1}$	1.70 - 15.50	19.43	24.33	16.33	15.10	16.36
Neutrophil	%	20.00 - 36.40	22.00	42.40	14.00	15.30	27.40
Limfosit	%	96.00 - 112.40	78.70	49.30	59.10	80.10	66.30
Monosit	%	6.00 - 21.00	15.40	8.30	7.60	6.30	6.40

Table 3. Histology Score Status

Treatment	Before Treatment		After Biofloc treatment	
	Length of villi (µm)	Width of villi (µm)	Length of villi (µm)	Width of villi (µm)
P0	260.80	920.70	422.70 ^{a} ± 60.3	961.43 ^{a} ± 26.1
P1			580.00^{b} ± 13.0	1707.21^{b} ± 13.4
P2			631.90^{c} ± 9.9	2431.42^{c} ± 11.3
P3			500.80^{b} ± 49.9	919.60^{a} ± 75.9

Note: The letters behind the numbers indicate a significant difference (p<0.01).

The leukocyte value in all treatments was above the normal value. The high leukocyte value was caused by the high stocking density, which can cause friction between fish in the container. The production of white blood cells in large numbers indicates that the fish has a physiological disorder [19]. Stress due to high stocking density can cause health problems and the immune system and inhibit the growth of the fish's body. Therefore the body will automatically produce more leukocytes than usual. It is the fish body's first defense mechanism [20].

Histological

The results of intestinal histological observation during the study are presented in Table 3. In accordance with Table 3, the highest value was presented by treatment P2, which in villi length shows a value of 631.90 and villi width of 2431, while the lowest value in villi length is shown by treatment P0, which is 422.70. Furthermore, the lowest villi area value was shown by treatment P3 which is 919.6. The length and width of the villi affect nutrient absorption in the intestine as a metabolism process. If there is good absorption of nutrients, there will be maximum utilization of feed, and of course, there will be good growth and can increase production.

After Biofloc Treatment

Figure 1A before biofloc treatment showed that the intestinal villi are shorter and narrower. The absorption process of nutrients in the intestine will be inhibited compared to Figure 1B after biofloc treatment, where the intestinal villi are longer and wider. The length and width of the villi are strongly influenced by probiotic bacteria, namely *Lactobacillus heterochiochii* and *Bacillus* sp., which develop and move in the intestine to decompose and absorb nutrients in the intestine. The provision of probiotics in fish farming systems can increase the growth of cultured organisms because probiotics can stimulate the intestine to secrete digestive enzymes, increase the immune response in the host, and inhibit the growth of pathogenic bacteria [21].

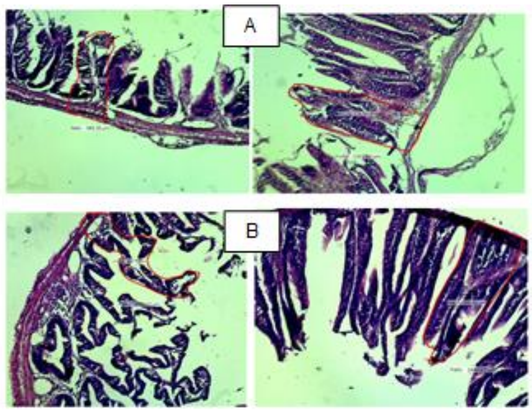

Figure 1. Intestinal histology of villi; A. Villi before biofloc treatment, B. Villi after biofloc treatment.

CONCLUSION

From the research data, the best dose of probiotics for petrofish was 1.5ml.L-1 of water. It can grow optimal flocks and improve water quality and natural food through high-protein flocs.

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